CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-567

MICROBIOLOGY REVIEW

DIVISION OF ANTIVIRAL DRUG PRODUCTS (HFD-530)
MICROBIOLOGY REVIEW
NDA: 21-567 **SN**: 000 **DATE REVIEWED:** 3/3/03
Microbiology Reviewer: Lisa K.Naeger, Ph.D.

Sponsor's Name and Address:

Bristol-Myers Squibb
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Reviewer's Name(s): Lisa K. Naeger, Ph.D.

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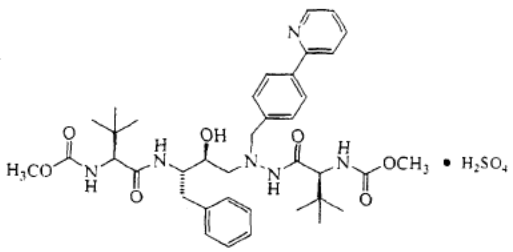
Related/Supporting Documents: IND56,897 (BMS), (b) (4) (b) (4) DMF No. (b) (4), DMF No. (b) (4), IND56,897 SN-425, NDA21,567 B1 and B2

Product Name(s): Atazanavir (BMS-232632, ATV)
Proprietary: Reyataz™
Non-Proprietary/USAN: Atazanavir sulfate
Code Name/Number: BMS-232632

Chemical Name: Dimethyl (3S, 8S, 9S, 12S)-9-benzyl-3,12-di-tert-butyl-8-hydroxy-4,11-dioxo-6-[4-(2-pyridyl)benzyl]-2,5,6,10,13-pentaazaetradecanedioate sulfate (1:1)

Structural Formula:



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Atazanavir (BMS-232632, ATV)
Molecular Formula: $C_{38}H_{52}N_6O_7 \bullet H_2SO_4$
Molecular Weight: 704.9

Dosage Form(s): 100/150/200 mg capsule
Route(s) of Administration: Oral
Pharmacological Category:
Indication(s): Treatment of HIV infection in combination with other antiretroviral agents

Dispensed: Rx X OTC_____

Abbreviations: α-1AGP, alpha-1 acidic glycoprotein; APV, amprenavir; ARV, antiretroviral; ATV, atazanvir; CC, cytotoxic concentration; CI, combination index; d4T, stavudine; DC, discontinuation; ddI, didanosine; DNA, deoxyribonucleic acid; HAART, highly active antiretroviral therapy; HIV, human immunodeficiency virus; IC, inhibitory concentration; IDV, indinavir; LOQ, Limit of Quantification; LPV, lopinavir; moi, multiplicity of infection; mAb, monoclonal antibody; NFV, nelfinavir; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, non-nucleoside reverse transcriptase inhibitor; PBMCs, peripheral blood mononuclear cells; PCR, polymerase chain reaction; PI, protease inhibitor; pol, polymerase; PPT, polypropylene tubes; PR, protease; RNA, ribonucleic acid; RT, reverse transcriptase; RTI, reverse transcriptase inhibitor; RTV, ritonavir; TLOVR, Time to Loss of Virologic Response; TP, triphosphate; SQV, saquinavir; WT, wild-type

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Executive Summary

This original NDA-21567 describes a novel protease inhibitor, atazanavir (ATV), for the treatment of HIV infection in combination with other antiretroviral agents formulated in 100, 150 and 200 mg capsules. Atazanavir is an azapeptide HIV-1 protease inhibitor that exhibits anti-HIV-1 activity with an EC_{50} of 2 to 5 nM against a variety of HIV-1 isolates in several cell-types.

There are currently seventeen FDA-approved anti-HIV drugs including six protease inhibitors (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir), seven NRTIs (abacavir, didanosine, lamivudine, stavudine, tenofovir, zalcitabine, and zidovudine), three NNRTIs (delavirdine, efavirenz and nevirapine), and one fusion inhibitor (enfuvirtide). To support ATV use in combination with other antiretroviral agents, the antiviral and cytotoxic effects of ATV in two-drug combinations were examined *in vitro* with all the approved anti-HIV drugs. Results from these studies indicated that the combination of ATV with abacavir and the NNRTIs: delavirdine, efavirenz, and nevirapine are additive-antagonistic. Combinations of ATV with the protease inhibitors (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir) and the NRTIs (didanosine, lamivudine, stavudine, tenofovir, zalcitabine, and zidovudine) showed additive antiviral activity interactions. Minimal to no cellular cytotoxicity was observed with any of these compounds alone or in combination with ATV.

To assess the potential for ATV resistance development and to identify amino acid changes associated with ATV resistance, the applicant utilized *in vitro* selection. Three HIV strains were passaged at increasing concentrations of ATV and resistant viruses were selected after 4-5 months at final ATV concentrations of 200-500 nM. These selected viruses exhibited 93- to 183-fold increases in ATV resistance, which is the change in the IC_{50} value compared to the wild-type parental strain. The key amino acid changes in the protease were I50L, which is different from the amprenavir-associated resistant mutation I50V, and I84V, A71V, and M46I, which are associated with resistance to other protease inhibitors.

The applicant has provided evidence that ATV resistance corresponds to the I50L and A71V mutations by constructing recombinant viruses from eight clinical isolates. These viruses with the I50L with or without the A71V mutation show 2- to 17-fold changes in their IC_{50} values for ATV compared to the wild-type parental strain. Furthermore, the addition of the I50L mutation results in replication-impaired viruses with a five-fold defect. The A71V mutation restores some viability to the virus suggesting it may be a compensatory mutation. Importantly, recombinant viruses containing the I50L mutation either with our without A71V remain susceptible to other protease inhibitors suggesting treatment-naïve patients who develop the I50L mutation in their virus would still have other treatment options.

Atazanavir-resistant isolates have been obtained from patients on atazanavir therapy. Fourteen ATV–resistant clinical isolates from trials of treatment-naïve subjects who were virologic failures developed the I50L mutation. Development of the I50L mutation ranged from 12-80 weeks of ATV therapy, averaging 50 weeks. An examination of the clinical isolates developing the I50L mutation showed an average 11-fold change from baseline for ATV and an increased susceptibility to approved protease inhibitors (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir) indicating that other protease inhibitors have activity against virus with the I50L mutation.

Mutations that developed in 36 isolates that were ATV-resistant and virologic failures from the trials of treatment-experienced subjects included A71V, I84V, L90M, N88S/D, M46I and I50L - all of which were observed in the *in vitro* selection experiments. These ATV-resistant isolates showed a median 12-fold change in ATV susceptibility and were highly cross-resistant with other protease inhibitors (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir). Four of the 36 isolates developed the I50L mutation on ATV treatment.

The response based on baseline genotype showed that if a virus had an I84V, L90M, A71V, N88S/D, or M46I mutation at baseline, the response to ATV treatment was not as effective as comparative treatments. Furthermore, ATV loses its effectiveness as clinical isolates become resistant to three or more protease inhibitors with > 80% of isolates resistant to 4 or 5 other protease inhibitors also resistant to ATV.

In summary, there are different possible resistance pathways for ATV. ATV has a unique pathway in treatment-naïve patients with the development of a key mutation, I50L. The I50L mutation is specific for ATV resistance and is the predominant mutation developing in antiretroviral therapy-naïve patients. Importantly, viruses with the I50L mutation remain susceptible to the other approved protease inhibitors. The other pathway occurring in treatment-experienced patients follows a common PI-resistance pathway with the development of mutations associated with resistance to multiple protease inhibitors. Mutations L90M, I84V, N88, and A71V/T appear to confer ATV resistance and reduce clinical response to ATV. The evidence suggests that if other protease inhibitor mutations are present, ATV resistance develops primarily through the later pathway rather than the I50L pathway. Finally, if isolates are resistant to three or more protease inhibitors, they are more likely to be ATV resistant.

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1. Recommendations

1.1. Recommendation and Conclusion on Approvability

This NDA for atazanavir 100, 150 and 200 mg capsules is approvable with respect to microbiology for the treatment of HIV in combination with other anti-HIV agents. It offers a simple once-a-day regimen with the benefit of reduced lipid and triglycerides compared to lopinavir. ATV has a unique resistance profile in antiretroviral-naïve patients with the development of the I50L mutation, which retains susceptibility to other protease inhibitors (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir). In antiretroviral-treatment-experienced patients, the use of ATV should depend on ATV susceptibility determined by genotypic and/or phenotypic assays. ATV is highly cross-resistant with other protease inhibitors against HIV-1 isolates from treatment-experienced patients.

1.2. Recommendation on Phase 4 (Post-Marketing) Commitments, Agreements, and/or Risk Management Steps, if Approvable.

1. Submit analysis of protease cleavage sites in ATV- resistant patients from studies 034, 043 and 045 by 1Q04.

2. Follow a cohort of patients who failed on ATV treatment and developed the I50L mutation on new physician-selected PI regimens for 48 weeks compared to an NNRTI-failure/PI-naïve patient cohort and determine treatment response, baseline genotypes and phenotypes, and genotypes and phenotypes of virologic failures. Protocol should be submitted by 1Q04.

3. Test the antiviral activity *in vitro* of atazanavir against multiple isolates of HIV-2 and non-clade B subtypes of HIV-1.

2. Summary of OND Microbiology Assessments

2.1. Brief Overview of the Microbiological Program

2.1.1. Non-clinical

Atazanavir (ATV, BMS-232632) is an azapeptide HIV-1 protease inhibitor that exhibits anti-HIV activity with an EC_{50} value of 2 to 5 nM against a variety of HIV isolates grown in cell cultures of PBMCs, macrophages, CEM-SS, and MT-2 cells. ATV specifically and selectively blocks the cleavage of the viral Gag and Gag-Pol precursor proteins in HIV-infected cells preventing viral particle maturation. Cytotoxicity with ATV is observed at concentrations >5,000-fold higher than that required for anti-HIV activity. Drug combination studies using

ATV with abacavir and NNRTIs demonstrated additive-antagonism and with NRTIs and PIs demonstrated additive effects.

HIV-1 resistant to ATV was selected from *in vitro* selection experiments in three different HIV-1 strains. These ATV-resistant HIV-1 isolates showed a 93- to 183-fold decrease in susceptibility to atazanavir compared to parental wild-type virus. Genotypic analyses indicated that I50L, A71V, N88S/D and I84V substitutions appeared to be key changes with possible roles in ATV resistance. Direct evidence for a role of the I50L mutation in ATV resistance was obtained by constructing recombinant viruses with the protease gene from clinical isolates. ATV resistance corresponded to the presence of the I50L and A71V mutations in the protease coding sequence. Results showed that the I50L mutation, sometimes combined with A71V and other changes, appears to be a signature substitution for ATV and mediates increased susceptibility to other PIs by an unknown mechanism. Clinical isolates resistant to one or two currently approved PIs (the majority nelfinavir-resistant with D30N mutations) were generally susceptible to ATV. Assessment of clinical isolates resistant to one or more PIs in patients never exposed to ATV showed that susceptibility to ATV decreased as the level of cross-resistance to other PIs increased.

2.1.2. Clinical Microbiology

Genotypic and phenotypic evaluation of clinical isolates from ATV-treated patients designated as virologic failures with decreased ATV susceptibility demonstrated that ATV displayed different resistance patterns depending on the patient population. When ATV was used in patients with no previous antiretroviral experience, clinical isolates developed a unique I50L mutation frequently accompanied by an A71V change. The I50L mutation resulted in ATV resistance, impaired viral growth, and increased susceptibility to other PIs. In contrast, isolates from treatment-experienced patients treated with ATV and ATV/SQV generally did not develop the I50L mutation but acquired several additional amino acid changes including I84V, L90M, M46I and N88S/D. These additional mutations in protease also conferred cross-resistance to the other approved PIs (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir). A significantly higher percentage of the clinical isolates from ATV treatment arms with PI mutations I84V, L90M, A71V, M46I and N88S/D at baseline were virologic failures compared to isolates from other treatment arms. This suggests that these mutations in the HIV-1 protease are detrimental to ATV antiviral activity and may affect the virologic response to ATV treatment clinically.

3. Administrative

3.1. Reviewer's Signature(s)

_____
[Lisa K. Naeger, Ph.D.]
Microbiologist, HFD-530

3.2. Concurrence

HFD-530/Signatory Authority _____Signature _____Date
HFD-530/Micro TL _____Signature _____Date

3.3. CC Block

OND Microbiology Review

1. Introduction and Background

1.1. Important Milestones in Product Development

CGP73547 was synthesized as an azapeptide inhibitor of the HIV protease and studies were performed at Ciba-Geigy with this compound to assess the inhibition of HIV-1 protease and antiviral activity against HIV-1. Subsequently, this compound was obtained by BMS from Novartis and then synthesized by BMS (BMS-232632).

1.1.1. Methodology

HIV RNA ANALYSIS

The generally accepted endpoint of virologic response in HIV clinical studies is the proportion of subjects with HIV RNA levels below the limit of quantification of 400 copies/mL or 50 copies/mL at a specified time point. Currently, the most widely used assay to measure HIV RNA with the polymerase chain reaction (PCR) technique is the Roche Amplicor HIV-1 Monitor^{TM} test version 1.0 (in the US) and version 1.5 (in Europe).

A substudy of 634 paired samples (322 on ATV; 312 on EFV) by the sponsor evaluated the virologic response at Week 48 on paired specimens analyzed with

Version 1.0 and Version 1.5 of the Roche Amplicor HIV-1 RNA Monitor assay using Ultra Sensitive methodology. Version 1.5 generally yielded higher HIV RNA levels than Version 1.0 in the 50 – 400 copies/mL range. Higher response rates were observed with Version 1.0 than Version 1.5, +7% for lower limit of quantification (LOQ) equals 400 copies/mL and +12% for LOQ equals 50 copies/mL. Additionally, within geographic regions, the magnitude of difference between response rates for Version 1.0 and 1.5 varied greatly with largest differences observed in Europe.

Specimen collection, storage and transport
Qualified central laboratories were used for the entire ATV clinical program to assay HIV RNA. Throughout the ATV clinical program, all patient specimens for a given study were shipped in the same way to central laboratories either at ambient temperature (Phase II) or frozen (Phase III).

In the Phase II clinical program (studies A1424007, A1424008 and A1424009), clinical sites were instructed by these laboratories to centrifuge blood samples for HIV RNA analysis and ship the centrifuged tube intact at ambient temperature to the lab for analysis. The Phase II program started in March 1999 and in late 2000 sites were given the option to ship protocol required repeat samples frozen or use frozen shipping at any time, but the sponsor states that this was rarely done.

Although, the appropriateness of ambient shipping for PPT tubes had been documented with the manufacturer, and the literature, during the Phase II program, it was noted that some ambient-shipped plasma specimens received by the central labs had evidence of hemolysis, suggestive of contamination of the plasma layer by red blood cells (and presumably other formed elements of the blood). As hemoglobin can inhibit PCR reactions, and contamination with white blood cells and platelets can lead to release of cell-associated viral particles into the plasma, it was determined that the accuracy and reproducibility of the RNA polymerase chain reaction (PCR) analyses would be enhanced, and variability diminished by shipping the PPT tubes frozen on dry ice. As a result, while the Phase II program continued to ship PPT tubes at ambient temperature to avoid changing procedures during the study, the entire Phase III program was designed to ship PPT tubes frozen to the central labs. Thus frozen shipping was used in studies A1424034, A1424043 and A1424045.

For the Phase II studies and the initial Phase III study, A1424034, the specifications provided by the central laboratories for processing the blood samples for HIV RNA analysis included instructions to centrifuge the PPT tubes of serum per the package insert for the tubes (b) (4) These specifications differ from those suggested by the Roche Amplicor RNA PCR kit (800 to 1600 g for 20 minutes). During the Phase III program it was noted that some sites in study A1424034 were spinning for more than the required (b) (4)

minutes. In the interest of enhancing uniformity for the sites and minimizing any factors that could contribute to variability, sites participating in the Phase III studies were given the clarification to centrifuge at 800 to 1600 g for 20 minutes as per the PCR kit. This clarification was made by modifications to laboratory manuals. There are no data to suggest that there is any substantive difference in separation efficiencies for these overlapping specifications.

In summary, throughout the ATV clinical program, all patient specimens for a given study were shipped in the same way to central labs either at ambient temperature (Phase II) or frozen temperature (Phase III). The minor adjustment in centrifugation duration that was implemented in the Phase III program was intended to provide uniformity for somewhat conflicting procedures but was unlikely to impact separation efficiencies. This modification would be expected to impact all treatment regimens in a given study in a comparable manner.

(b) (4) **PhenoSense**

PhenoSense is a recombinant phenotypic drug susceptibility assay for HIV that utilizes recombinant vectors to determine drug susceptibility of HIV from patient plasma or serum samples to protease and RT inhibitors. (b) (4)

he percent inhibition of viral replication is determined by comparing luciferase activity in the presence and absence of drug and IC_{50} values are determined from inhibition curves. The data are expressed as the ratio of the IC_{50} value obtained for mutant HIV-1 compared to the IC_{50} for the reference strain NL4-3. For performance assessment of ATV, two reference viruses, a drug sensitive and a reduced drug susceptibility reference vector referred to as CNDO and MDRC4, respectively, were used. CNDO is a "wild-type" control vector with PR and RT regions from laboratory strain pNL4-3. MDRC4 has numerous resistance-associated mutations in the PR and RT coding regions with varying degrees of reduced drug susceptibility to each of the antiretroviral drugs tested in the assay. The mean IC_{50} value for ATV from 152 CNDO runs was 1.1 nM with a range of 0.89 – 1.7 nM with values varying less than 2-fold. The mean IC_{50} value for ATV from 125 MDRC4 runs was 7 nM with a range of 5.2 – 9.4 nM with values varying less than 2-fold. The mean fold-change in ATV susceptibility of MDRC4 compared to CNDO was 6.2-fold.

Geneseq HIV Assay
Geneseq is a genotypic drug susceptibility assays utilizing DNA sequence analysis to detect mutations in HIV-1 PR and RT genes associated with resistance to RT and PR inhibitors. Geneseq utilizes the dideoxynucleotide chain termination method of DNA sequencing and cycle sequencing. (b) (4) Mutations are identified by comparing the amino acid sequence of the patient virus to the sequence of the NL4-3 reference virus. Custom Geneseq HIV software (b) (4) is used for analysis of the sequence files.

Both Geneseq and PhenoSense assays were performed (b) (4)

1.1.2. Prior FDA Microbiological Reviews

IND56897 Serial #029
Reviewer: N. Battula, Ph.D.
Date reviewed: August 19, 1999

Recommended the use of the UltraSensitive Roche Amplicor HIV-1 Monitor for the determination of plasma HIV-1 RNA in clinical trials.

IND56897 Serial #374
Reviewer: Lisa K. Naeger, Ph.D.
Date Reviewed: Jan.15, 2003

Recommendations:

1. Sponsor is advised to provide *in vitro* combination activity analyses for drug interactions of atazanavir with all approved anti-HIV agents.

2. The cross-resistance profile of atazanavir should be provided showing the cross-resistance of atazanavir with isolates resistant to other approved PIs. In addition, information should be provided showing the activity of all approved PIs against isolates resistant to atazanavir.

3. Sponsor has stated that isolates with mutations I50L or I50L/A71V, associated with atazanavir resistance, have enhanced susceptibility to other PIs. Please be advised that statistically significant data from multiple

isolates supporting increased susceptibility will be required if sponsor wishes to include such a statement in the label.

1.1.3. Major microbiological issues that arose during product development.

1. A number of studies suggest that Version 1.5 used on the same samples results in higher quantification of HIV RNA than Version 1.0 by up to 0.5 $\log_{10}$ copies/mL. A substudy of 634 paired samples (322 on ATV; 312 on EFV) by the sponsor evaluated the virologic response at Week 48 on paired specimens analyzed with Version 1.0 and Version 1.5 of the Roche Amplicor HIV-1 RNA Monitor assay using Ultra Sensitive methodology. Version 1.5 generally yielded higher HIV RNA levels than Version 1.0 in the 50 – 400 copies/mL range. Therefore, higher response rates were observed with Version 1.0 than Version 1.5, +7% when the lower limit of quantification (LOQ) equaled 400 copies/mL and +12% when the LOQ equaled 50 copies/mL. Additionally, within geographic regions, the magnitude of difference between response rates for Version 1.0 and 1.5 varied greatly with largest differences observed in Europe. However, since the same assays were used in the comparative arms, this should not affect the comparison between treatment arms.

2. Some ambient-shipped plasma specimens received by the central labs had evidence of hemolysis, suggestive of contamination of the plasma layer by red blood cells (and presumably other formed elements of the blood). As hemoglobin can inhibit PCR reactions, and contamination with white blood cells and platelets can lead to release of cell-associated viral particles into the plasma, it was determined that the accuracy and reproducibility of the RNA polymerase chain reaction (PCR) analyses would be enhanced, and variability diminished by shipping the PPT tubes frozen on dry ice. As a result, while the Phase II program continued to ship PPT tubes at ambient temperature to avoid changing procedures during the study, the entire Phase III program was designed to ship PPT tubes frozen to the central labs. Thus frozen shipping was used in studies A1424034, A1424043 and A1424045. Therefore, throughout the ATV clinical program, all patient specimens for a given study were shipped in the same way to central labs either at ambient temperature (Phase II) or frozen temperature (Phase III).

3. The sponsor was advised on January 15, 2003 to provide *in vitro* combination activity analyses for drug interactions of atazanavir with all approved anti-HIV agents and the cross-resistance profile of atazanavir with isolates resistant to other approved PIs (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir).

1.2. State of antimicrobials used for the indication (s) sought*:*

An estimated 40 million people were infected with HIV worldwide and 3 million died from AIDS in 2001. Since HAART regimens have been introduced, the number of AIDS cases decreased dramatically. HAART does not eradicate HIV from patients completely and even though the number of HIV RNA copies is reduced to undetectable levels, HIV re-emerges quickly after discontinuation of HAART. Therefore, with the currently available regimens, it is likely that most HIV-infected patients will require antiretroviral therapy throughout their lives.

There are currently seventeen FDA-approved anti-HIV drugs including six PIs (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir), seven NRTIs (abacavir, didanosine, lamivudine, stavudine, tenofovir, zalcitabine, zidovudine), three NNRTIs (delavirdine, efavirenz, nevirapine) and the fusion inhibitor T20 (enfuvirtide). NRTIs mimic nucleosides and target HIV-1 RT by competing with natural deoxynucleoside triphosphates for binding to RT and incorporation into newly synthesized viral DNA resulting in chain-termination. NNRTIs inhibit HIV-1 RT by binding near the catalytic site of RT and acting as noncompetitive inhibitors. PIs work at the late stage of viral replication to prevent virus production from infected cells. They block the HIV protease enzyme, which is necessary for the production of mature virions, resulting in defective particles that are unable to infect new cells. T20 (enfuvirtide) is a gp41 fusion inhibitor that was recently approved for the treatment of HIV.

Unfortunately, HIV develops resistance to antiretroviral drugs over time usually from the accumulation of multiple mutations. HAART regimens are also associated with acute toxicities such as diarrhea, kidney stones, rash, CNS toxicities and hepatotoxicity. Long-term toxicities from antiretroviral therapies include mitochondrial toxicities associated with NRTIs (lactic acidosis, myopathy, neuropathy, pancreatitis) and disorders of lipid metabolism (dyslipidemia) and glucose metabolism (lipodystophy, hypercholesterolemia, hypertriglyceridemia) associated with PIs. These tolerability issues make compliance to therapy more challenging. Compliance is an important determinant of successful virologic suppression for patients on HAART. Regimens that are well-tolerated and easy to administer with a few pills once daily are likely to aid in patient compliance and improve clinical outcomes. There is a need for new anti-HIV drugs that are well-tolerated and easy to use as well as with new modes of action and low likelihood of viral resistance development.

2. Non-clinical Microbiology

2.1. Mechanism of Action

BMS-232632 is an azapeptide inhibitor of HIV-1 protease and has activity against recombinant HIV-1 protease in an *in vitro* fluorescence-based peptide cleavage assay.

(b) (4)

In this assay, BMS-232632 has a K_i value of 0.75 nM which is comparable to the K_i values of indinavir (0.73 nM), nelfinavir (1.05 nM), ritonavir (1.01 nM), and saquinavir (0.39 nM).

Inhibition of HIV-1 proteolytic cleavage in virus-infected cells results in the accumulation of gag precursor product p55. Chronically-infected RF/H9 cells were treated with various concentrations of BMS-232632. The polyprotein p55 and the processed p24 product from virions secreted from BMS232632-treated cells were analyzed by Western blotting using a monoclonal antibody (mAb) that recognized both p55 and p24. BMS-232632 blocked the processing of HIV-1 precursor protein p55 to p24 in HIV-infected cells in a dose-dependent manner with an EC_{50} value of 47 nM.

The activity of BMS-232632 on HIV-1 protease in comparison with several human aspartic proteases was analyzed by an amino acid peptide substrate assay using a fluorescent substrate. BMS-232632 is specific for HIV-1 protease with an IC_{50} value of 1 nM compared to IC_{50} values of >10,000 nM for the human aspartic proteases renin, cathepsin E, cathepsin D, pepsin, and gastricsin (Table 1, Study 910068964, Volume 1-1a, page 12).

The ability to select for protease mutants under ATV drug selection pressure provides *in vivo* validation that ATV targets the HIV-1 protease protein.

Table 1. Effect of BMS-232632 (CGP 73547) on several human aspartic proteases.

Enzyme	IC_{50} (nM)
HIV-1 Protease	1
Renin	>10,000
Cathepsin E	>10,000
Cathepsin D	>10,000
Pepsin	>10,000
Gastricsin	>10,000

2.2. Antiviral Activity

CGP 73547 was synthesized as an azapeptide inhibitor of HIV-1 protease and studies were performed at Ciba-Geigy with this compound to assess the inhibition of HIV-1 proteases and antiviral activity against HIV-1. Subsequently, this compound was

obtained by BMS from Novartis (company resulting from Ciba-Geigy merger) and then synthesized by BMS (BMS-232632).

In vitro antiviral activity of BMS-232632 was initially analyzed using an RT replication assay with laboratory strain HIV-1/MN/H9 at a multiplicity of infection of 0.14 in MT-2 cells. From this data, the EC_{90} and EC_{50} values for BMS-232632 were determined to be 3.9 and 1.3 nM compared to 36.2 and 9.8 nM for indinavir and 19.5 and 4.2 nM for saquinavir, respectively.

Antiviral assays performed in primary human lymphocytes determined that suppression of HIV-1 replication below the limit of detection was achieved with 30 nM of BMS-232632 and that the EC_{90} value was 8.1 nM.

Antiviral evaluation of atazanavir using a variety of HIV-1 strains (clinical isolate 006, laboratory strains RF and LAI, and the macrophage-tropic virus BaL) at a multiplicity of infection of 0.005 in several host cell-types (PBMC, CEM-SS, MT-2 and macrophages) showed that this compound has an EC_{50} value of 2 - 5 nM (Table 2, Study 910068968, Volume 1-1A, page 9).

2.3. Antiviral Activity in the presence of human serum and α-1 acid glycoprotein

Most HIV protease inhibitors are significantly protein bound which reduces the effective concentration. Therefore, it is important to evaluate the effect of α-1 acid glycoprotein (AGP) and 40% human serum on the activity of atazanavir. The antiviral activity of BMS-232632 was examined in the presence of 2 mg/mL of α-1 AGP (induced concentration) and compared to the antiviral activity of saquinavir, indinavir, VX-478, and SC52151 in MT-2 cells and primary human lymphocytes (Table 3 and Table 4, Study 910068964, Volume 1-1a, pages 16 and 17).

Table 2. Comparative Anti-HIV Activity of Atazanavir

HIV-1/Host cell	Compound	EC_{50} (nM)	CC_{50} (μM)[a]	Selectivity Index
Clinical isolate 006/PBMC[b]	Atazanavir	2.62 ± 0.45	50	19,084
	Indinavir	5.00 ± 0.65	ND	ND
	Nelfinavir	6.14 ± 0.21	> 10	> 1629
	Ritonavir	43.1 ± 13.4	60	1392
	Saquinavir	24.8 ± 4.23	> 40	> 1613
	Amprenavir	54.3 ± 1.14	> 100	> 1842
RF/MT-2[c]	Atazanavir	3.89 ± 0.35	27.9	7172
	Indinavir	16.2 ± 2.22	> 5	> 309
	Nelfinavir	25.9 ± 2.33	> 5	> 193
	Ritonavir	69.8 ± 10.8	ND	ND
	Saquinavir	13.4 ± 1.19	ND	ND
	Amprenavir	53.4 ± 5.35	ND	ND
RF/CEM-SS[a]	Atazanavir	4.85 ± 1.17	45.8	9443
	Indinavir	13.3 ± 2.54	> 5	> 376
	Nelfinavir	17.9 ± 5.02	> 5	> 279
	Ritonavir	35.5 ± 9.75	ND	ND
	Saquinavir	5.28 ± 1.21	ND	ND
	Amprenavir	16.6 ± 3.92	ND	ND
LAI/MT-2[c]	Atazanavir	5.28 ± 0.77	27.9	5284
	Indinavir	28.7 ± 4.28	> 5	> 174
	Nelfinavir	23.4 ± 5.46	ND	ND
	Ritonavir	129 ± 25.5	ND	ND
	Saquinavir	12.1 ± 2.18	ND	ND
	Amprenavir	39.8 ± 5.96	ND	ND
Bal/Macrophage[c]	Atazanavir	4.38 ± 1.49	47	10,731
	Indinavir	26.1 ± 7.21	ND	ND
	Nelfinavir	25.1 ± 5.58	ND	ND
	Ritonavir	191 ± 83.85	ND	ND
	Saquinavir	17.2 ± 3.41	ND	ND
	Amprenavir	48.3 ± 13.5	ND	ND

Viruses: 006: clinical HIV isolate; RF and LAI: T-cell tropic HIV; Bal: macrophage tropic HIV.
ND Not Determined.
[a]Cytotoxicity endpoints were XTT staining: antiviral assay for RF/CEM-SS was also XTT.
[b]Antiviral endpoint: p24.
[c]Antiviral endpoint: RT.

Table 3. Effect of α-1 acid glycoprotein (AGP) on the EC_{90} values (MT-2/HIV-1MN) of several HIV-1 protease inhibitors

HIV-1 protease inhibitor	EC_{90} (nM) No α-1 AGP	EC_{90} (nM) + α-1 AGP	Fold increase EC_{90}
BMS-232632	3.9	30.7	8
Saquinavir	7.7	145.7	19
Indinavir	12.5	65	5
VX-478	22.7	406.7	18
SC 52151	273	4685	17

Table 4. Effect of α-1 acid glycoprotein (AGP) on the EC_{90} values (lymphocytes/HIV-1 LAV) of several HIV protease inhibitors

HIV-1 protease inhibitor	EC_{90} (nM) No α-1 AGP	EC_{90} (nM) + α-1 AGP	Fold increase EC_{90}
BMS-232632	8.1	81.8	10
Saquinavir	18.7	173.2	9
Indinavir	34	112	3
VX-478	30	3363	112
SC 52151	300	Not reached	

This concentration of α-1 AGP diminished the anti-HIV activity of atazanavir by 8-fold against HIV-1 MN in MT-4 cells and 10-fold against HIV-1 LAV in lymphocytes, comparable to the reduction in anti-HIV activity of indinavir and saquinavir in the presence of α-1 AGP.

EC_{50} values were determined in the presence and absence of 40 % human serum from HIV-1 RF infection of CEM-SS cells four days post-infection using an RT assay. This concentration of human serum diminished the anti-HIV activity of atazanavir by five-fold, similar to the reduction in anti-HIV activity of saquinavir, indinavir, ritonavir and amprenavir in the presence of human serum (Table 5, Study 910068968, Volume 1-1A, page 11). Nelfinavir had the greatest reduction (17-fold) in antiviral activity in the presence of serum.

ATV is 86% protein bound and the addition of 40% human serum to the conventional cell protection assay decreased potency five-fold. The trough level (C_{min}) observed using the once daily 400 mg dosing regimen of ATV was determined to be > 200 ng/mL (280 nM; > 8-fold higher than the protein-adjusted EC_{50} for ATV using a conventional multi-cycle cell infection assay and significantly higher (40-fold) than the EC_{50} determined [redacted] (b) (4) using a single cycle assay.

Table 5. Effect of Human Serum on HIV RF Replication in CEM-SS Cells

	EC_{50} (nM)			
Inhibitor	**10% FCS[a]**	**40% HS**	**Fold Increase in EC_{50}**	**Relative EC_{50}[b] in Human Serum**
Atazanavir	1.5	7.8	5	1
Nelfinavir	9.0	150	17	19
Saquinavir	7.8	25	3	3
Indinavir	5.1	20	4	3
Ritonavir	47	140	3	18
Amprenavir	31	64	2	8

FCS: fetal calf serum: HS: human serum.
[a]The values are different from that in Table 1 because of normal experimental variation.
[b]Ratio of EC_{50} values of each protease inhibitor in the presence of human serum to that observed for atazanavir, which was arbitrarily set at one.

2.4. Cytotoxicity

BMS-232632 did not show cytotoxic activity up to 30 μM in a XTT assay in MT-2 cells. Cytotoxicity determinations of protease inhibitors by XTT staining in PBMCs, MT-2, CEM-SS, and macrophages revealed that the selective index of atazanavir (5,284 –19,084) compares favorably with those of other protease inhibitors (See Table 2 above).

2.5. Metabolites of BMS-232632

BMS-232632 is the major circulating component in human plasma. Two metabolites of BMS-232632, BMS-421419 and BMS-551160, each constituting approximately 10% of plasma radioactivity have been identified in the systemic circulation following administration of radioactive BMS-232632 to humans. These metabolite compounds were evaluated against T-tropic HIV-1 RF and NL4-3 of MT-2 cells and M-tropic (SF-162 or Bal) infection of PM1 cells using RT assays and p24 assays with BMS-232632 as a control. BMS-421419 did not exhibit anti-HIV activity up to 200 nM while BMS232632 had inhibitory activity against HIV-1 RF, NL4-3 and SF-162 with EC_{50} values of 3.2, 1.3 and 0.74 nM, respectively. BMS-551160 did not exhibit anti-HIV activity up to 1000 nM while BMS232632 had inhibitory activity against HIV-1 RF, NL4-3 and Bal with EC_{50} values of 1.5, 1.1 and 1.8 nM, respectively.

These metabolites were also evaluated for cytotoxicity in both MT-2 cells and PM1 cells using XTT assays. The observed CC_{50} values of BMS-421419 were 312 and 254 μM in MT-2 and PM1 cells, respectively. The observed CC_{50} values of BMS-551160 were 201 and 122 μM in MT-2 and PM1 cells, respectively.

2.6. *In Vitro* Drug Combination Studies

Analysis of drug combination effects.
Experiments were performed in PBMCs infected with a clinical isolate utilizing p24 assays. Checkerboard dilution of drugs in two-fold steps in an 8X6 checkerboard and Prichard and Shipman's MacSynergy program were used to evaluate the drug combination studies (Prichard, 1992; Prichard, 1993). This method evaluates the combined effects of drugs in volumes ($\mu M^2\%$). A positive volume indicates synergy while a negative volume indicates antagonism. Values between +50 and –50 are considered additive. Values between +50 and +100 or –50 to –100 are interpreted as moderately synergistic or antagonistic, respectively. Volumes greater than +100 or less than –100 indicate strong drug interactions. The drug interactions were also evaluated using the method of Chou and Talalay (1984) and Chou and Rideout (1991), which compares a constant-ratio mixture of the two drugs to the monotherapies by calculating a combination index (CI). CI values of <1, =1, >1 indicate synergism, additivity and antagonism, respectively. Results for drug combinations of BMS-232632 with NRTIs are shown in Table 6 and with PIs in Table 7 (Study 910068967, Volume 1-1A, pages 8 and 10). Taking all the CI values and the MacSynergy analyses into account, the two-drug combinations of BMS-232632 with these four RT inhibitors and five PIs resulted in overall additive to weak-synergistic anti-HIV effects. No significant antagonism resulted even at the highest concentrations of drug combinations tested. In addition, no enhanced cytotoxicity was detected with any drug combination as measured by XTT reduction assay. Additional drug combination results examining the combination of ATV with abacavir, lopinavir, tenofovir, zalcitabine, and the three NNRTIs (delavirdine, efavirenz, and nevirapine) were submitted in NDA 21-567 N000/B1 and are shown in Table 8. To assess the effects of these drug combinations, combination indices (CI) were calculated according to Chou and Rideout (1991). The results showed additive antiviral interactions for ATV combined with lopinavir, tenofovir, and zalcitabine with CI values =1 or <1 and no cytotoxicity. The CI values for abacavir and the NNRTIs were >1 at multiple dilutions and inhibition levels. All combination indices were tested for departure from additivity using isobologram methods (Gessner, 1998). Theoretically, additivity is implied if the CI is equal to one, synergy if the CI <1, and antagonism if the CI is >1. However, the noise inherent in the dose-response curves propagates to the estimates that are obtained from these curves. The uncertainty about the estimates needs to be taken into consideration when assessing the drug combination effect. Therefore, asymptotic response curves means less certainty in the estimates and translates to larger confidence intervals. These intervals are used to test for departure from additivity by comparing the bound to one – a lower bound of

the interval greater than 1 indicates antagonism, an upper bound of less than 1 indicates synergism and a value of 1 contained in the interval indicates additivity. Using this criteria, the sponsor considers abacavir, delavirdine, efavirenz, and nevirapine additive even though their CI values are > 1. However, the CI consistently falls above 1 at different inhibition levels and this would suggest an antagonistic interaction and certainly the CI values above 2 suggest antagonism. Furthermore, the large confidence intervals can position the data to be inconclusive. Therefore, an additive-antagonistic claim for the combination of ATV with abacavir, delavirdine, efavirenz, and nevirapine seems more reasonable.

Table 6. Combination Antiviral Activity of ATV with HIV Reverse Transcriptase Inhibitors.

Drug Combined with BMS-232632	MacSynergy Volume (μM²%)		CI values at % HIV Inhibition			Overall Result
	Synergy	Antagonism	Molar ratio	50%	70%	
stavudine	0.78	-5.4	1: 5 1: 40	0.90 0.98	0.82 0.80	additive
didanosine	16.6	-1.2	1: 150 1: 600	0.93 1.00	0.82 0.92	additive
zidovudine	44.3	0	2: 1 1: 2	0.73 0.87	0.76 0.72	additive
lamivudine	11.2	0	1: 1.25 1: 5	0.95 0.91	0.97 0.91	additive

Table 7. Combination Antiviral Activity of ATV with HIV Protease Inhibitors.

Drug Combined with BMS-232632	MacSynergy Volume (μM²%)		CI values at % HIV Inhibition			Overall Result
	Synergy	Antagonism	Molar ratio	50%	70%	
ritonavir	31.4	-36.7	1: 42 1: 83	1.21 1.05	1.12 1.08	additive
indinavir	75.5	-41.7	1: 2.5 1: 8.3	0.87 1.00	1.08 0.94	additive
saquinavir	42.1	-2.7	1.3: 1 1: 3	1.02 0.86	1.13 0.89	additive
nelfinavir	5.7	-0.78	1: 3.1 1: 12.5	1.43 1.07	0.96 0.94	additive
amprenavir	0	-10.8	1: 3.75 1: 15	1.34 1.37	1.08 0.95	additive

Table 8. Anti-HIV Activity from Two Drug Combinations with ATV

Molar Ratio (EC_{50} ratio)[a]	Combination Indices at % HIV Inhibition (Confidence Interval)[b] 50%	75%	90%	Overall Result
Tenofovir				
0.5:1 (1:1)	**0.71** (0.53, 0.88)	**0.66** (0.43, 0.89)	**0.79** (0.36, 1.21)	
0.2:1 (1:2.5)	**0.84** (0.62, 1.06)	**0.64** (0.40, 0.87)	**0.63** (0.26, 1.00)	**Additive-Synergistic**
1.25:1 (2.5:1)	**0.93** (0.73, 1.12)	**0.76** (0.50, 1.02)	**0.72** (0.34, 1.10)	
Zalcitabine				
0.02:1 (1:1)	**1.02** (0.00, 2.71)	**0.79** (0.00, 3.24)	**0.64** (0.00, 4.09)	
0.008:1 (1:2.5)	**0.75** (0.64, 0.86)	**0.98** (0.78, 1.19)	**1.31** (0.89, 1.74)	**Additive-Synergistic**
0.05:1 (2.5:1)	**0.89** (0.80, 0.98)	**0.95** (0.82, 1.09)	**1.07** (0.84, 1.31)	
Abacavir				
0.01:1 (1:1)	**0.82** (0.64, 0.99)	**1.04** (0.73, 1.36)	**1.39** (0.73, 2.04)	
0.004:1 (1:2.5)	**0.74** (0.60, 0.87)	**1.02** (0.76, 1.28)	**1.45** (0.86, 2.03)	**Additive-Synergistic**
0.025:1 (2.5:1)	**0.87** (0.70, 1.03)	**1.02** (0.74, 1.30)	**1.24** (0.71, 1.78)	
Delavirdine				
0.1:1 (1:1)	**1.00** (0.78, 1.22)	**1.25** (0.86, 1.63)	**1.61** (0.84, 2.38)	
0.04:1 (1:2.5)	**1.24** (0.87, 1.61)	**1.41** (0.82, 2.00)	**1.63** (0.56, 2.71)	**Additive**
0.25:1 (2.5:1)	**1.34** (0.96, 1.71)	**1.28** (0.78, 1.77)	**1.26** (0.49, 2.02)	
Efavirenz				
10:1 (1:1)	**1.17** (0.81, 1.52)	**1.62** (0.93, 2.31)	**2.25** (0.75, 3.76)	
4:1 (1:2.5)	**1.14** (0.75,1.53)	**1.64** (0.86, 2.43)	**2.37** (0.60, 4.15)	**Additive**
25:1 (2.5:1)	**1.00** (0.65, 1.35)	**0.90** (0.46, 1.34)	**0.81** (0.19, 1.42)	
Nevirapine				
0.05:1 (1:1)	**1.27** (0.93, 1.61)	**1.43** (0.90, 1.96)	**1.64** (0.69, 2.59)	
0.02:1 (1:2.5)	**1.34** (1.03, 1.65)	**1.47** (0.99 1.95)	**1.63** (0.80, 2.46)	**Additive**
Lopinavir				
0.5:1 (1:1)	**1.07** (1.02, 1.12)	**1.03** (0.86, 1.20)	**0.99** (0.69, 1.30)	
0.2:1 (1:2.5)	**1.26** (0.76, 1.75)	**1.05** (0.88, 1.23)	**0.88** (0.80, 0.96)	**Additive**
1.25:1 (2.5:1)	**1.27** (0.98, 1.56)	**1.16** (0.95, 1.36)	**1.05** (0.85, 1.25)	

a Ratio of atazanavir to comparator compound

b A lower bound of the asymptotic confidence interval greater than 1 indicates antagonisms, an upper bound of less than 1 indicates synergism and a value 1 being contained in the interval indicates additivity

3.0 *In vivo* Studies

3.1. Efficacy

The primary efficacy endpoint in study 034 was the percentage of patients with HIV RNA levels below the limit of quantification of 400 copies/mL at 48 weeks. The primary efficacy endpoint for study 043 was the magnitude of viral suppression as assessed by the change from baseline in plasma HIV RNA levels (expressed in $\log_{10}$) through 24 weeks. Multiple secondary analyses were performed for each study.

The following two tables summarize efficacy results for selected trials. The first table provides efficacy results for atazanavir 400 mg in studies 007, 008, and 034. In these studies, atazanavir was similar to efavirenz and nelfinavir in a Time to Loss of Virologic Response (TLOVR) analysis using both 400 copies/mL and 50 copies/mL as limits of detection.

At 24 weeks in study 043, subjects receiving atazanavir had a mean decrease of 1.73 $\log_{10}$ copies/mL as compared to a mean decrease of 2.16 $\log_{10}$ copies/mL for lopinavir/ritonavir patients. The time-averaged difference (TAD) estimate (ATV - LPV/RTV) for the change from baseline in HIV RNA level through 24 weeks was 0.31 $\log_{10}$ copies/mL (97.5% CI: 0.06, 0.55), favoring lopinavir/ritonavir.

Preliminary efficacy results at 16 weeks of a limited number of enrolled subjects in study 045 were provided in this NDA. A ritonavir-boosted dose of atazanavir 300 mg appeared to be similar to LPV/RTV, each given with tenofovir and an optimized NRTI. Atazanavir given in combination with saquinavir appeared to be inferior.

Please refer to reviews of the medical reviewer, Kendall Marcus, and the statistical reviewer, Tom Hammerstrom, for more detailed efficacy analyses.

Summary of Efficacy Treatment-Naïve Studies
Time to Loss of Virologic Response (TLOVR)

	Study 034		Study 007		Study 008	
HIV RNA	**ATV AZT/3TC**	**EFV AZT/3TC**	**ATV ddI/d4T**	**NLF ddI/d4T**	**ATV d4T/3TC**	**NLF d4T/3TC**
	Number of Subjects/Total (%)					
< 400 copies/mL	281/404 (70)	258/401 (64)	48/78 (62)	50/82 (61)	123/181 (68)	54/91 (59)
< 50 copies/mL	131/404 (32)	150/401 (37)	26/78 (33)	23/82 (28)	60/181 (33)	35/91 (38)
TAD_{48}	-2.67	-2.74	-2.42	-2.33	-2.51	-2.31

Summary of Efficacy – Treatment-Experienced Studies
Time to Loss of Virologic Response (TLOVR)

	Study 043 - 24 weeks		Study 045 – 16 weeks		
HIV RNA	**ATV 2 NRTIs**	**LPV/RTV 2 NRTIs**	**ATV 300 RTV 100 TNF/NRTI**	**ATV 400 SQV 1200 TNF/NRTI**	**LOP RTV TNF/NRTI**
	Number of Subjects/Total (%)				
< 400 copies/mL	69/114 (61)	93/115 (81)	21/37 (57)	17/34 (50)	21/35 (60)
< 50 copies/mL	47/114 (41)	60/115 (52)	14/37 (38)	10/34 (29)	7/35 (20)
TAD_{16}	---	---	-1.74	-1.70	-1.87
TAD_{24}	-1.73	-2.16	---	---	---

3.2. Safety

Three areas of concern emerged during the atazanavir development program. The first is the frequency of hyperbilirubinemia seen in atazanavir-treated subjects. The hyperbilirubinemia is dose dependent and appears to be due to inhibition of UDP-glucuronosyl transferase, an enzyme responsible for the conjugation of bilirubin. Over three-fourths of all patients experienced an elevation of bilirubin while on treatment. The hyperbilirubinemia observed in atazanavir-treated subjects was predominantly indirect, regardless of the degree of hyperbilirubinemia observed. The hyperbilirubinemia seen during the development program of atazanavir did not appear to result in an increased incidence of hepatotoxicity relative to selected PIs or to efavirenz.

The second safety issue relates to effects of atazanavir on the QT and PR interval. A placebo-controlled pharmacokinetic study designed to evaluate effects of

atazanavir on ECG parameters revealed a dose-dependent prolongation of the QT interval. Prolongation that may be considered a signal for increased risk for development of Torsades de Pointes (TdP) was seen at a dose of 800 mg given once daily. During evaluation of the effects of atazanavir on the QT interval it was also found that atazanavir produced dose-dependent prolongation of the PR interval. The incidence of first degree AV block was common and occurred in over 50% of subjects receiving 800 mg of atazanavir. In summary, while pharmacokinetic studies revealed moderate effects of atazanavir on the PR interval, clinical events related to prolongation of the PR interval were rare. First degree AV block was the most common abnormality observed. Effects on the QT interval at the proposed dose appeared to be minimal.

The final safety issue relates to lipid metabolism. It was noted during phase 2 studies of treatment-naïve subjects that treatment with nelfinavir resulted in greater increases in lipid parameters relative to atazanavir and this finding was confirmed in phase 3 studies of both treatment-naïve and treatment-experienced patients. In general, atazanavir produced a significantly lower change in total cholesterol, fasting LDL, and triglycerides than all comparators.

Please refer to review of the medical reviewer, Kendall Marcus, for a more detailed safety analysis.

3.3. Pharmacodynamics

The IC_{50} of ATV in the presence of 40% human serum was determined to be 7.8 nM, 5-fold greater than the IC_{50} without serum. The trough level (C_{min}) observed using the once daily 400 mg dosing regimen of ATV was determined to be > 200 ng/mL (280 nM). Adjusting the ATV IC_{50} values (2-5 nM) for protein binding (5-fold) gives an inhibitory quotient (c_{min}/protein adjusted IC_{50}) range of 11 to 28. This range should give good coverage to the majority of "wild-type" isolates that presumably exist in ARV-naïve subjects. However, the median fold change of isolates with resistance to ATV was approximately 11-fold. This suggests isolates from antiretroviral-experienced subjects that may be resistant to ATV or develop ATV resistance may not be covered adequately by the c_{min} of ATV. Please refer to the review of the pharmacokinetics reviewer, Jenny Zheng, for a detailed analysis of the pharmacokinetics and pharmacodynamics of ATV.

4.0 Resistance Studies

4.1 Resistance

To assess the potential of atazanavir resistance development, the RF, LAI and NL4-3 strains of HIV-1 were serially passaged in culture in the presence of increasing concentration of drug starting at 2X the EC_{50} value for up to 4.8 months. Supernatants were periodically collected for drug susceptibility assays

and cell pellets were harvested for DNA sequence analysis (Table 9, Study 930002291, Volume 1-1A, page 10).

Table 9. Drug Susceptibility of Atazanavir Resistant Viruses

Virus Strains	Selection Time (Months)	Selection Drug Conc. (nM)	Major Protease Substitutions	Fold Resistance[a]
RF	1	25	N88S	4
	2.4	100	M46I, N88S	6
	3.5	225	V32I, M46I, A71V, N88S	12
	4.8	500	V32I, L33F, M46I, A71V, I84V, N88S	183
LAI	2.6	28	L10Y/F, I50L, A71V, N88S	36
	4.7	500	L10Y/F, I50L, L63P, A71V, N88S	93
NL4-3	3.9	40	V32I, M46I, I84V	6
	4.6	200	V32I, M46I, I84V, L89M	96

[a] Fold resistance was found to be significant at the ≤ 0.05 level by using an unpaired *t* test. Results were derived from more than 9 experiments for RF and 4 - 5 experiments for LAI and NL4-3, respectively.

The RF strain of HIV-1 showed a six-fold decrease in susceptibility to atazanavir following 2.4 months of passage in drug concentrations up to 100 nM. Virus variants of the LAI strain emerged at 2.6 and 4.7 months and displayed 36- and 93-fold decreases in susceptibility to atazanavir, respectively. After 3.9 months of selection, the NL4-3 variant showed 6-fold reduction, but after 4.6 months had a 96-fold reduction in atazanavir susceptibility compared to WT.

Genotypic and phenotypic analyses indicated that N88S substitution appeared first during the ATV-selection process in the RF and LAI viruses. An I50L substitution appears as a key change in the LAI virus, while the I84V substitution seems to have an important role in the RF and NL4-3 viruses.

For comparative purposes, the RF strain was also passaged in parallel with clinically available HIV protease inhibitors. Selection with nelfinavir resulted in an RF virus that exhibited 9-fold nelfinavir resistance after 1 month of selection and 35-fold resistance after 2 months of nelfinavir treatment. A ritonavir-resistant HIV-1 RF virus emerged after 3 months of selection with a 71-fold change in

susceptibility to ritonavir. In this one experiment, ATV resistance developed more slowly than nelfinavir or ritonavir, and similarly to indinavir and ampenavir (data not shown).

Direct evidence for a role of the I50L mutation in ATV resistance was obtained by constructing recombinant viruses with the protease gene from eight clinical isolates. ATV resistance corresponded to the presence of the I50L and A71V mutations in the protease coding sequence with a mean 12-fold change [range 2.9 –17] in ATV susceptibility from baseline (Table 10, Volume 1-1, Report 930003058, page 29). Phenotypic profiling of these recombinant viruses with ATV and six other PIs further showed that resistance is ATV specific and coincided with enhanced susceptibility of the other PIs (Table 10). These results showed that the I50L mutation, sometimes combined with A71V and other amino acid changes, appears to be a signature substitution for ATV and mediates increased susceptibility to other PIs by an unknown mechanism.

To further demonstrate the specific impact of the I50L substitution on ATV resistance, the I50L and A71V substitutions were inserted alone or in combination into the wild-type protease gene of three recombinant viral clones: LAI, NL4-3 and RF. Two- to five-fold decreases in ATV susceptibility were observed in all three viral backbones containing the I50L substitution that increased from 6- to 10-fold with the combination of I50L plus A71V. Increased susceptibility to amprenavir, indinavir, nelfinavir and ritonavir was observed in all three viral backbones containing the I50L mutation either alone or in combination with A71V (Table 11, Volume 1-1, Report 930003058, page 30). These results provided further evidence that the I50L and/or A71V substitutions placed in a variety of viral backbones resulted in decreased ATV susceptibility. Moreover, enhanced susceptibility to amprenavir, indinavir, nelfinavir and ritonavir was observed in all three viral backbones containing either I50L change alone or in the combination of I50L and A71V substitutions (Table 11). While it is unclear at this time how the I50L-mediated increase in PI susceptibility measured *in vitro* will translate to patients clinically, it is apparent that ATV-resistant virus containing the I50L mutation appear to retain susceptibility to other currently marketed PIs.

Table 10. Phenotype and genotype of recombinant viruses containing an I50L substitution.

Recom. Virus	Baseline/ Treatment	EC_{50} fold change vs. reference strain							Amino acid substitutions
		ATV	APV	IDV	NFV	RTV	SQV	LPV	
810024	baseline	**7.6**	1.4	2.3	**> 142**	0.8	nd	nd	10F, 13V, 23I, 30N, 37S, 41K, 62V, 63P, 70R, 71T, 88D, 93L
	ATV	**22**	0.1	0.1	**6**	0.1	nd	nd	10F, 13V, 30N, 37S, 41K, ***50L***, 62V, 63P, 70R, 71T, 88D, 93L
050 100	baseline	na	na	na	na	na	na	na	na
	ATV	**31**	0.02	0.1	1.1	0.05	0.3	0.08	***50L***, 63P, 70R, ***71V***, 88S
010 020	baseline	na	na	na	na	na	na	na	na
	ATV	**2.5**	0.04	0.04	0.3	0.1	0.4	0.1	46I, ***50L***, 63P, 69Q, 77I, 93L
020 398	baseline	0.8	1.2	2.4	**4.2**	1.4	0.8	0.9	35D, 36L, 37S, 41K, 57K, 62V, 63P, 93L
	ATV	**12**	0.9	0.6	1.0	0.2	0.08	0.2	35D, 36L, 37S, 41K, ***50L***, 57K, 62V, 63P, 93L
020 347	baseline	1.1	1.3	1.4	1.5	0.9	0.6	1.3	13V, 35D, 63P
	ATV	**19**	0.7	0.7	1.2	2.2	0.25	0.5	13V, 35D, 36I, ***50L***, 63P, ***71V***, 82A
045 296	baseline	0.6	0.5	0.3	0.8	0.5	0.5	0.7	63P, 77I, 93L
	ATV	**9.5**	0.4	0.2	0.6	0.1	0.1	0.06	45K, ***50L***, 63P, ***71V***, 73S, 77I, 93L
031 372	baseline	1.5	0.6	0.4	1.1	0.9	0.8	1.4	10I, 63A, 93L
	ATV	**13**	0.2	0.5	1.6	0.3	0.5	**2.5**	10I, ***50L***, 63P, 73S, 93L
040 450	baseline	0.9	1.6	1.1	1.5	0.9	0.7	1.1	19I, 41K, 69Y
	ATV	**10**	0.6	0.5	1.2	0.2	0.1	0.1	19I, 41K, ***50L, 71V***, 74S

I50L and A71V signature substitutions were highlighted in bold and italicized.

Bold and underline indicated those with fold change ≥ 2.5-fold.

nd: not done; na: sample not available

Table 11. Recombinant viruses with I50L/A71V substitutions

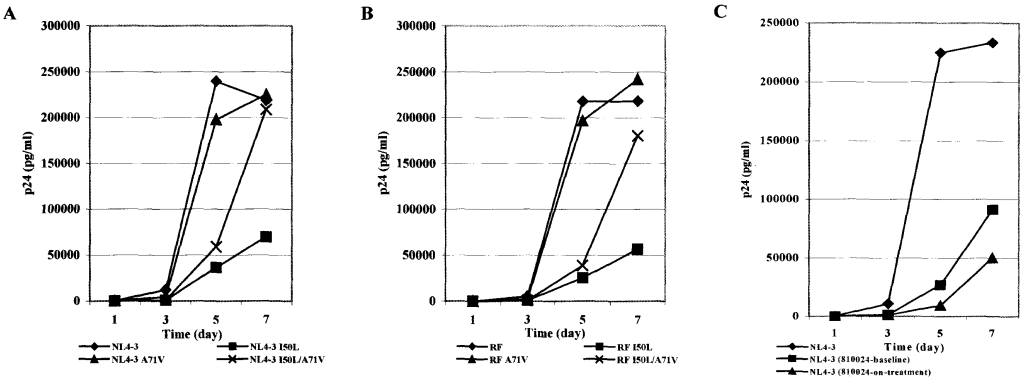
Virus	EC_{50} (nM) (fold change)				
	ATV	NFV	IDV	RTV	APV
LAI	1.5	10.8	29.9	51.6	39.5
I50L	**8.2 (5.4)**	2 (0.2)	3.5 (0.1)	3.6 (0.1)	8.8 (0.2)
A71V	3.7 (2.4)	11.4 (1.1)	18.8 (0.6)	25.5 (0.5)	28.9 (0.7)
I50L/A71V	**15.1 (10)**	3.5 (0.3)	8.8 (0.3)	1.4 (0.2)	12.3 (0.2)
NL4-3	1.5	5.5	15.1	26.9	33.8
I50L	3.1 (2)	0.5 (0.1)	1.4 (0.1)	0.7 (0.1)	4.6 (0.1)
A71V	2.4 (2)	7.2 (1.3)	14.5 (1.0)	19.7 (0.7)	11.1 (0.3)
I50L/A71V	**8.6 (6)**	1.8 (0.3)	4.0 (0.3)	4.2 (0.2)	7.8 (0.2)
RF	0.7	2.5	8.9	9.5	15.2
I50L	2.2 (3)	0.2 (0.1)	0.9 (0.1)	0.6 (0.3)	4.1 (0.3)
A71V	0.6 (0.9)	3.6 (1.4)	10.5 (1.2)	22.5 (2)	9.1 (0.6)
I50L/A71V	**4.0 (6)**	0.5 (0.2)	1.2 (0.1)	1.4 (0.2)	3.8 (0.3)

Bold and underline indicated those with resistance fold change ≥ 2.5-fold.

Effect of the I50L substitution on Viral Replication.

The growth curves of wild-type and recombinant viruses containing I50L or I50L/A71V mutations were determined. Insertion of the I50L substitution in either NL4-3 or RF protease backbones resulted in significantly impaired viruses (Figure 1, Volume 1-1, Report 930003058, page 31). The addition of the A71V change with I50L restores some viability. The I50L substitution may be unfavorable to protease activity resulting in a weakened HIV-1 while the A71V replacement is likely to be compensatory substitution.

Figure 1. Growth curves of recombinant viruses containing I50L and A71V substitutions.



4.2 Protease Cleavage Sites

Changes in the protease P7/P1 and P1/P6 cleavage sites can occur during treatment with HIV protease inhibitors. The gag sequence spanning the P7-P1-P6 junctions from clones obtained during passage of all three viral strains were examined. One ATV-induced change was found in the highly-resistant RF viruses at the P1/P6 site (F/LQSR**P** to F/LQSR**L**). All 12 RF clones that displayed 183-fold ATV resistance and 7/11 RF clones that had 12-fold ATV resistance contained this specific P1/P6 change. The LAI viruses with 36- and 93-fold resistance to ATV contained a 12-amino acid deletion near the P1/P6 cleavage site. The significance of these ATV-associated substitutions at the cleavage sites to ATV clinical resistance remains to be determined and is a Phase IV commitment.

5.0 Cross-Resistance

5.1 *In Vitro* Cross-Resistance

To assess whether ATV is effective against HIV-1 variants resistant to other PIs, viral strains resistant to five approved PIs (amprenavir, indinavir, nelfinavir, ritonavir and saquinavir) were generated *in vitro* and assayed (Table 12, Study 930002291, Volume 1-1a, page 17). Six-to nine-fold changes in ATV susceptibility were observed with viruses containing the I84V mutation, which also contributes in varying degrees to amprenavir, indinavir, nelfinavir, ritonavir and saquinavir resistance. The amprenavir and saquinavir-resistant viruses and one of the ritonavir-resistant viruses retained susceptibility to ATV. From this set of experiments, cross-resistance of ATV with other PIs appears variable.

For reciprocal cross-resistance studies, ATV-resistant viruses selected by *in vitro* selection experiments were analyzed by susceptibility testing against five approved protease inhibitors (Table 13, Study 930002291, Volume 1-1a, page 19). The highly ATV-resistant RF 0.05 virus exhibiting 183-fold ATV resistance and containing the mutations I84V and A71V as well as other mutations was cross-resistant to amprenavir, indinavir, nefinavir, and ritonavir, but retained susceptibility to saquinavir. The LAI viruses containing the I50L and A71V mutations and conferring high-level ATV resistance (36- and 93-fold) were still susceptible to amprenavir, indinavir, ritonavir and saquinavir with a moderate decrease in nelfinavir susceptibility (5-fold).

Table 12. Cross-Resistance Profile of HIV-1 Isolates Resistant to PIs

Selection Drug (μM)	Major Substitutions	Fold Resistance[a]					
		ATV	NFV	IDV	RTV	SQV	APV
RF-IDV (4.0)	V32I,M46I,V82F,I84V	9[a]	8[a]	24[a]	72[a]	1.4	27[a]
Clinical[b]-IDV	L10R,M46I,L63P,V82T,I84V	6[a]	6[a]	15[a]	21[a]	6[a]	4[a]
RF-RTV (5.0)	M46I,V82F,I84V,L90M	7[a]	22[a]	29[a]	71[a]	8[a]	28[a]
Clinical[c]-RTV	L10I,L63A,V82F	0.5	5	3	11	0.3	2
RF-NFV (2.0)	D30N,M46I	3[a]	35[a]	3	1	1	2
RF-APV (5.0)	V32I,M46I,I47V,V82I	2	11[a]	11[a]	16[a]	1	82[a]
NL-SQV[d]	L10I,G48V,L90M	1	1.5	3	2	8[a]	2

[a] Fold resistance was found to be significant at the ≤ 0.05 levels using an unpaired t-test. Results were derived from 4 experiments for RF-IDV (4 μM), Clinical-IDV and RF-NFV (2 μM) and from 2 experiments for NL-SQV and RF-APV (5 μM).

[b] Clinical isolate obtained (b) (4)

[c] Clinical isolate obtained (b) (4)

[d] Recombinant virus in the NL4-3 background.

Table 13. Cross-Resistance Profile of Atazanavir-Resistant Viruses to Approved Protease Inhibitors

Virus (Selection Conc. [μM])	Major Substitutions	Fold Resistance					
		ATV	NFV	IDV	RTV	SQV	APV
RF (0.23)	V32I,M46I,A71V,N88S	12[a]	12[a]	4[a]	3[a]	2[a]	0.4[a]
RF (0.50)	V32I,L33F,M46I, A71V,I84V,N88S	183[a]	21[a]	36[a]	54[a]	2[a]	9[a]
NL4-3 (0.04)	V32I, M46I, I84V	6[a]	2[a]	2	24[a]	1	2[a]
NL4-3 (0.20)	V32I,M46I,I84V,L89M	96[a]	8[a]	9[a]	71[a]	2[a]	51[a]
LAI (0.028)	L10Y,I50L,A71V,N88S	36	1	1	0.06	1	0.1
LAI (0.50)	L10Y,I50L,L63P, A71V,N88S	93[a]	5[a]	2	0.1[a]	2[a]	0.15[a]

[a] Fold resistance was found to be significant at the ≤ 0.05 levels using an unpaired t-test. Results were derived from > 9 experiments for RF, and 4 - 5 experiments for LAI and NL4-3, respectively.

5.2 Cross-Resistance of Clinical Isolates

Phenotype of I50L and I50V-containing isolates.

The cross-resistance relationship between atazanavir and amprenavir was examined because the substitutions at amino acid I50, *i.e.* I50L and I50V, are signature resistance changes for atazanavir and amprenavir, respectively. The phenotypes of a panel of viruses containing either an I50L or I50V were determined (Table 14, Volume 1-1, Report 930003058, page 33). Results showed no cross-resistance. Viruses containing an I50L were specifically resistant to atazanavir while viruses with an I50V mutation were specifically resistant to amprenavir.

Table 14. Phenotype of I50L and I50V-containing isolates.

		Fold Change	
Clinical Isolates	**Residue Change**	**ATV**	**APV**
034 043 226	I50L	3.5	0.3
007 010 020	I50L	4.9	0.6
034 094 772	I50L	5.2	0.5
007 049 450	I50L	7.9	0.4
034 076 586	I50L	8.6	0.6
007 020 398	I50L	8.8	0.8
008 060 076	I50L	9.5	0.6
007 045 296	I50L	9.9	0.3
007 020 347	I50L	11	0.4
044 060 169	I50L	12	0.7
020 810024	I50L	26	0.7
020 810016	I50L	36	0.8
045 154 562	I50V	0.6	1.3
045 087 094	I50V	0.3	3.5
045 013 025	I50V	0.4	4.2
11557.2	I50V	0.6	8
045 083 103	I50V	1.2	9.4
045 109 280	I50V	0.4	11
V216965	I50V	0.8	11
V33778	I50V	0.5	12
045 051 001	I50V	1.5	13
045 150 398	I50V	13	112
V213888	I50V	0.5	14
045 003 096	I50V	0.9	23

ATV Susceptibility Profile of 551 Clinical Isolates Resistant to Other PIs

The PI susceptibility profile of 950 HIV-1 clinical isolates [sixty (b) (4) and an additional 890 from the screening process for clinical studies 009, 043, and 045] was evaluated. Of these isolates, 551 displayed susceptibility levels > 3-fold higher than reference strain (NL4-3 (b) (4) and HXB2 (b) (4)) to at least one PI. ATV susceptibility is retained against > 80% of isolates resistant to 1-2 PIs with the majority of these isolates resistant to nelfinavir with the D30N mutation. There is clear trend toward loss of ATV susceptibility as isolates become resistant to three or more PIs with ATV sensitivity retained against only 5% of isolates resistant to five PIs (Table A). The median fold change in ATV susceptibility for isolates resistant to 1-5 PIs is 1.6, 2.1, 4.0, 6.2 and 22.0, respectively (Table B).

Table A. ATV Susceptibility Against PI-Resistant Clinical Isolates (n = 551)

PI	Number of Isolates Resistant to 1 PI (n = 157)	Number of Isolates Resistant to 2 PIs (n = 57)	Number of Isolates Resistant to 3 PIs (n = 99)	Number of Isolates Resistant to 4 PIs (n = 96)	Number of Isolates Resistant to 5 PIs (n = 142)
ATV	19 (12%)	11 (19%)	65 (66%)	81 (84%)	135 (95%)
SQV	7 (4%)	13 (23%)	39 (39%)	61 (64%)	142 (100%)
IDV	0 (0%)	6 (11%)	22 (22%)	28 (29%)	31 (22%)
RTV	27 (17%)	48 (84%)	97 (98%)	96 (100%)	142 (100%)
NFV	121 (77%)	38 (67%)	85 (86%)	95 (99%)	142 (100%)
APV	2 (1%)	6 (11%)	16 (16%)	36 (38%)	142 (100%)

Number of resistant isolates (%)

Table B. Median fold-change in ATV susceptibility by Number of Marketed PIs that Isolates are Resistant To

Number of Marketed PIs that Isolates are Resistant To	Median fold-change in ATV susceptibility
1	1.6
2	2.1
3	4.0
4	6.2
5	22.0

6. **Clinical Microbiology**

The clinical development program consisted of 15 clinical studies including:

- Studies with antiretroviral treatment-naïve subjects
  Phase II – 007 (48 wk, n = 420) and 008 (48 wk, n = 467)
  Phase III – 034 (48 wk, n = 810)
- Studies with antiretroviral treatment-experienced subjects
  Phase II – 009 (48 wk, n = 85)
  Phase III – 043 (24 wk, n = 229)
- Studies with antiretroviral highly treatment-experienced subjects
  Phase III – 045 (24 wk, n = 106)
- Two roll-over studies
  007 and 009 rolled-over to 041
  008 rolled-over to 044
- One pediatric study - 020

Baseline and on-treatment viral samples (where available) were obtained for resistance analyses from patients who experienced virologic failure while enrolled in

- AI424007 – (ATV +d4T + ddI in treatment-naïve subjects)
- AI424041 – (rollover from 007 and 009, ATV vs. NFV or RTV in PI-experienced subjects)
- AI424008 – (ATV + d4T +3TC in treatment-naïve subjects)
- AI424044 – (rollover from 008, ATV + d4T + 3TC in PI-experienced patients)
- AI424034 – (ATV vs. EFV in combination with ZDV+3TC, treatment-naive)
- AI424009 – (ATV/SQV + 2 NRTIs in PI-experienced patients)
- AI424020 – (PACTG 1020)(ATV combination regimen in treatment-naïve and -experienced pediatric subjects)
- AI424043 – (ATV vs. LPV/RTV + 2 NRTIs in treatment-experienced subjects)
- AI424045 – (ATV/RTV vs. ATV/SQV vs. LPV/RTV + tenofovir + NRTI in highly treatment-experienced subjects)

Virologic Failures are defined as "Time to Loss of Virologic Response (TLOVR) which includes

1) Rebound: confirmed (two consecutive) plasma HIV RNA values greater than the Limit of Quantification (LOQ) after achieving confirmed level below LOQ during the treatment phase,
2) Never Suppressed: plasma HIV-1 RNA levels never achieve confirmed suppression with at least 48 weeks of randomized treatment,

3) Insufficient Viral Response: plasma HIV-1 RNA levels never achieve confirmed suppression and the investigator identifies the reason for treatment discontinuation prior to week 48 due to insufficient viral load response.

Subjects designated as virologic failures had paired plasma specimens from baseline and on-treatment evaluated for phenotypic and genotypic changes. Virologic failure specimens were selected for resistance evaluation only if they contained > 1000 copies/mL HIV-1 RNA and if confirmed that the specimen did not come from a patient at a time of transient non-adherence to study drugs. Isolates were genotyped by (b) (4) (GeneSeq™) and phenotyped by (b) (4) (PhenoSense™). The reference strain was NL4-3. ATV phenotypic resistance is defined as a fold change in susceptibility > 2.5.

6.1 ATV Clinical Resistance

The phenotype and genotype of 163 evaluable clinical isolates from patients on ATV-containing regimens who experienced virologic failure or discontinued before suppression from all studies 007, 008, 009, 034, 041, 044 and 043 were analyzed. Fifty (31%) of these isolates displayed ATV resistance with > 2.5-fold change in ATV susceptibility (Table C). These clinical isolates exhibited decreased susceptibility to ATV ranging from 2.8- to 141-fold (average 18.9; median 9.6). Of 96 clinical isolates from trials of treatment-naïve subjects, 14 (15%) were ATV-resistant. Of 67 isolates from trials 009 and 043 of treatment-experienced subjects, 54% were ATV-resistant.

Table C. Evaluable Clinical Isolates from Patients on ATV-containing Regimens who Experienced Virologic Failure or Discontinued Before Suppression

	All Studies 007, 008, 009, 034, 041, 044, and 043	**Naïve Trials 007, 008, 034**	**ARV-Experienced Trials 009, 041, 044, and 043**	**Study 045**
Evaluable isolates	163	96	67	17
Mean	6.6	2.6	12.2	22.6
Median	1.4	1.04	3.4	14.5
ATV >2.5-fold	50 (31%)	14 (15%)	36 (54%)	10 (59%)
Mean	18.9	10.6	21.0	35.7
Median	9.6	8.7	11.8	29.5

Fourteen clinical isolates from HIV-infected patients on ATV regimens from antiretroviral treatment-naïve trials 007, 008, 034 that had virologic failure and had >2.5-fold change in ATV susceptibility had an average of 10.6-fold and median of

8.7-fold in ATV susceptibility (range of 3-to 48-fold). All 14 isolates developed the I50L mutation. Four of these isolates developed the I50L mutation in roll-over studies 041 or 044, but the patients had been on ATV as the sole protease inhibitor from earlier trials 007 and 008. From studies 009, 041, 044, and 043 in antiretroviral treatment-experienced patients, 36 clinical isolates from ATV-containing regimens had virologic failure or discontinued before suppression and had a change in ATV susceptibility of >2.5-fold. These isolates had an average of 21-fold and median of 11.8-fold change in ATV susceptibility with a range of 2.8-to 141-fold.

At the time of this submission, study 045 utilizing ritonavir-boosted ATV was in progress and only the samples analyzed by week 16 were submitted late in the review cycle. The division has decided not to include this study in the review for traditional approval of this NDA. The sample size for evaluable isolates and virologic failures is very small in this analysis and may not be reflective of a final 045 analysis. The phenotype and genotype of evaluable clinical isolates from patients who experienced virologic failure or discontinued before suppression treated with ATV from study 045 (n= 17) demonstrated that 10 isolates (59%) had a >2.5-fold change in ATV susceptibility with an average of 36-fold and median 30-fold change in ATV susceptibility (range of 2.8-to 149-fold).

The percentage of virologic failures that exhibited ATV-resistance increased from 15% in trials of treatment-naïve subjects to $\geq$50% in trials of ARV treatment-experienced subjects.

Mutations Associated with ATV-Resistance in Naïve-trials

Of the clinical isolates that were virologic failures and ATV-resistant from the treatment-naïve trials 007, 008 and 034, 14 developed the I50L mutation and 9 of these also developed the A71V mutation (See Appendix 1). ATV resistance for the isolates containing the I50L averaged a 10.6-fold change in ATV susceptibility from the reference strain [I50L + A71V = 10.8-fold; I50L alone = 10.0-fold]. The timing of the development of the I50L mutation ranged between 12 and 172 weeks with a median of 57 weeks. Four of the isolates that developed the I50L mutation were from roll-over trials 041 and 044 and had been on prior ATV treatment in trials 007 and 008. Three isolates developed the M46I mutation concurrently with the I50L and/or A71V mutations. Three isolates that were ATV-resistant and from treatment-naïve trials did not develop the I50L mutation. Isolate PID 00046 00248 from study 007 (ATV, ddI, d4T) had ATV resistance of 48-fold and rebounded at week 24 with PR mutations I84V, L90M, A71V, E35D, M36I and RT mutations M41L, D67N, K103N, M184V, T215Y, L210W, and K219Q (no baseline genotype). Isolate PID 00045 00016 from study 007 had RT mutations M184V, D67N, and K70R and PR mutations E35D and M36I at rebound week 64 (no baseline genotype), and isolate 00114 00065 from a patient who never responded to treatment in study 034 (ATV, AZT, 3TC) had RT mutations M184V, T69N, and K70R and PR mutations D30N and

A71V at baseline (2.2-fold change in ATV susceptibility) and developed M46I/M at week 41 (3-fold change in ATV susceptibility). Given the extent of the mutations present in these three isolates, it is likely that these isolates were from patients who were either not ARV-naïve or were infected with resistant virus strains.

Twenty-one ATV-resistant isolates from studies 007/041, 009/044, 034 and 020 had an I50L substitution. Genotypic analysis of isolates revealed that 15 isolates developed the unique substitution I50L. There are six additional isolates from studies 007/41, 008/44, 020 and 034 that contained evidence of the I50L substitution. While phenotypic data are not available for study 007 isolates 031 372 and 050 100, study 008/044 isolate 069 362 and study 020 isolate 810016, each had the I50L substitution emerge in the "on-treatment" isolate sample. In addition, two isolates from study 034 (052 517 & 084 441) displayed no phenotypic evidence of ATV resistance, but evidence of I/L or I/V mixtures at residue 50. This brings the total number of ATV treatment isolates with evidence of the I50L change to 21. Of these 21 isolates, 9 (43%) also contained evidence of an A71V substitution.

The appearance of an I50L substitution occurs infrequently when compared to the other reported PI-related changes. However, a strong correlation appears to exist between the emergence of the I50L substitution in the trials of naïve subjects, resistance to ATV and increased susceptibility to other PIs. Additional support can be found with study 007 isolates 020 347 and 045 296, which both showed I50I/L + A71A/V mixtures prior to the appearance of the I50L and A71V substitutions and development of significant ATV phenotypic resistance.

Resistance Phenotype of HIV Harboring the I50L Mutation for Other PIs

Phenotypic data for 10 ATV-resistant isolates that developed the I50L mutation on ATV treatment from the trials of treatment-naïve subjects and that had a baseline and post-baseline sample were analyzed. These isolates were susceptible to all PIs analyzed at baseline (Table D). The 10 isolates had an average 11-fold change in ATV susceptibility after the development of the I50L mutation but remained susceptible to the other PIs with fold changes from baseline of <1. This evidence suggests that patients on ATV treatment that develop the I50L mutation in their HIV will still have other PI treatment options.

Table D. Phenotype of ATV-Resistant Isolates that Developed the I50L Mutation In Naïve Trials (Matched Pairs)

	n	Baseline*	Post-Baseline*	Fold-change from Baseline	P value
ATV	10	0.87	9.43	**10.9**	0.004
APV	10	0.69	0.47	**0.69**	0.11
IDV	5	0.88	0.41	**0.47**	0.005
LPV	4	0.98	0.28	**0.28**	0.06
RTV	10	1.06	0.23	**0.22**	0.0007
SQV	10	0.75	0.34	**0.45**	0.000005
NFV	10	1.22	0.71	**0.59**	0.002

* Average fold-change from reference strain

Mutations Associated with ATV-Resistance in Treatment-Experienced Trials

There were 36 evaluable ATV-resistant clinical isolates that were virologic failures from the ATV treatment arms of trials 009, 041, 044 and 043 in antiretroviral-experienced patients (See Appendix 2). Four of the isolates developed the I50L mutation on ATV treatment. Eighteen of these isolates had evidence of the L90M mutation at baseline. One isolate from a patient on ATV/SQV treatment developed the L90M mutation by week 31 and had a 14-fold increase in ATV resistance from baseline. The M46I mutation developed concurrently with the L90M mutation in this isolate and I84V subsequently developed by week 56 leading to a 33-fold increase in ATV resistance from baseline. Five isolates from ATV/SQV treatment arms developed the I84V mutation and had an average 17-fold increase from baseline in ATV resistance. Three isolates from patients on ATV treatment as the only PI developed the I84V mutation with an average 5-fold change from baseline in ATV resistance. The M46I mutation also developed in the HIV of three patients on ATV/SQV treatment concurrently with the I84V and L90M mutations. Nine isolates developed the A71V or A71T mutation, four of which also developed the L90M or I84V concurrently on ATV/SQV treatment. Fifteen isolates had a substitution at N88 at baseline. Two isolates developed the N88S/D substitution on ATV treatment with an average 5-fold increase in ATV resistance from baseline. One isolate from ATV/SQV treatment arm developed the N88S mutation with A71V by week 33 with little change in ATV susceptibility from baseline. One patient on ATV/SQV treatment whose isolate had a N88D mutation at baseline was on prior nelfinavir therapy and had developed the D30N and M36L mutations that conferred a 4-fold change in ATV susceptibility.

The cross-resistance of the 32 isolates that were virologic failures and ATV-resistant from treatment-experienced patients from Trials 009 and 043 was analyzed (the four

isolates that developed the I50L mutation were excluded from this analysis). The median fold change in ATV susceptibility for these 32 isolates was 11. Thirty-seven percent of the isolates were resistant to amprenavir and 47% of the isolates were resistant to lopinavir with median fold changes in ATV susceptibility of 1.7 and 2, respectively. Greater than 80% of the isolates were resistant to indinavir, ritonavir, or saquinavir and all the isolates were resistant to nelfinavir with median fold changes in ATV susceptibility ranging from 5–28 (Table E).

Table E. Cross-Resistance of the Virologic Failure clinical isolates that were ATV-Resistant from Treatment-Experienced Patients in Trials 009 and 043

	ATV	APV	IDV	LPV	RTV	SQV	NFV
% of resistant isolates	100%	37%	91%	47%	87%	81%	100%
Mean fold-change in ATV susceptibility	22.9	2.7	10.3	14.1	26.7	25.6	54.7
Median fold-change in ATV susceptibility	11	1.7	7.8	2	7	5.6	28

(Virologic failures n = 32; n=11 for IDV and n=17 for LPV)

One isolate from treatment-experienced trial 043, patient number 00064 00173, developed the I50L mutation on ATV 400 mg and ddI/d4T treatment at week 16 and experienced viral load rebound and virologic failure. This patient had prior indinavir experience and the M184V mutation in their HIV detected in the baseline genotype. There is only baseline phenotypic data for this patient and it shows resistance to lamivudine and abacavir (note: indinavir was not tested).

Response Based on Baseline Phenotype

Baseline phenotypic analyses from studies 009 and 043 showed that 56% of the isolates showed resistance to at least one PI [52% with nelfinavir resistance], 74% of the isolates showing resistance to at least one NRTI [66% with resistance to lamivudine], and 20% showing resistance to efavirenz or nevirapine. At baseline, 24% of the isolates had resistance to ATV.

Of the 24% of isolates from studies 009 and 043 that were resistant to ATV (>2.5-fold) at baseline, 100% were also resistant to nelfinavir, 47% were resistant to indinavir, 43% were resistant to lopinavir, 62% resistant to ritonavir, and 59% were resistant to saquinavir (Table F).

Baseline phenotypic analysis from study 045 demonstrated that 54% of the isolates showed resistance to at least one PI [52% with nelfinavir resistance], 83% of the isolates showing resistance to at least one NRTI [74% with resistance to lamivudine], and 76% showing resistance to efavirenze or nevirapine. Overall 37% of the isolates had resistance to ATV.

Of the 37% of isolates from study 045 that were resistant to ATV, 62% of these isolates showed cross-resistance with amprenavir, 81% with lopinavir, 100% with NFV, 94% with ritonavir, and 78% with saquinavir (Table F).

Table F. Cross-Resistance of ATV-resistant isolates at Baseline (% of isolates resistant)

Trial	NFV	IDV	APV	LPV	RTV	SQV
009 and 043[1]	100%	47%	nd	43%	62%	59%
045[2]	100%	nd	62%	81%	94%	78%

[1] 24% of isolates from studies 009 and 43 were resistant to ATV at baseline
[2] 37% of isolates from study 045 were resistant to ATV at baseline

An analysis of response based on baseline phenotype showed that virologic failures in the ATV treatment arm from antiretroviral-experienced patient trials 009 and 043 had a 2.97-fold change in ATV susceptibility from reference significantly different from the 1.7-fold change in ATV susceptibility of the responders in the ATV treatment arm (Table G). In the highly antiretroviral-experienced patient trial 045, virologic failures in the ATV treatment arm had a 6.5-fold change in ATV susceptibility from reference which was significantly different from the 1.5-fold change in ATV susceptibility of the responders in the ATV treatment arm.

Table G. Response Based on Baseline Phenotype by Treatment Arm (Fold-change from reference)

	Treatment Naïve Patient Trials 007, 008, and 034		Antiretroviral-Experienced Patients Trials 009 and 043		Highly Antiretroviral Experienced Patient Trial 045	
	ATV	NFV or EFV	ATV	RTV/SQV or LPV/RTV	ATV	LPV/RTV
Responders	0.84	0.95	1.72	1.76	1.48	2.02
Virologic Failures	0.85	0.87	2.97	2.16	6.52	6.74
P value			0.02	0.24	0.001	0.06

Virologic failures includes discontinuations before suppression

Response Based on Baseline Genotype

Antiretroviral Experienced Patients Trials 009, 043, and 045
From trials of antiretroviral-experienced subjects, out of the 16 (44%) baseline isolates that contained the I84V mutation and were on ATV treatment, 15 (94%) were virologic failures or discontinued before suppression and 1 (6%) responded to treatment. This is in contrast to the 14 baseline isolates that contained the I84V mutations and received LPV/RTV or efavirenz treatment where 5 (36%) were virologic failures or discontinued before suppression and 6 (43%) responded (Table H). These 30 isolates containing the I84V mutation had an average 22.9-fold change and a median 13.8-fold change from reference in ATV susceptibility. Twenty-four (80%) of these I84V-containing isolates also contained an A71V or A71T mutation and had a 26.6 and 15.3 mean and median fold-change from reference in ATV susceptibility, respectively.

Table H. Response of Antiretroviral-Experienced Patients with the I84V or L90M Mutation at Baseline

	I84V[1]		L90M[2]	
	ATV	LPV/RTV or EFV	ATV	LPV/RTV, RTV/SQV or EFV
Virologic failures	7 (44%)	4 (29%)	43 (37%)	8 (12%)
DC before suppression	8 (50%)	1 (7%)	42 (37%)	20 (31%)
DC while suppressed		3 (21%)	7 (6%)	10 (15%)
Responders	1 (6%)	6 (43%)	23 (20%)	27 (42%)
n	16	14	115	65

[1]The 30 isolates containing the I84V mutation had an average 22.9 fold- change and a median 13.8 fold-change from reference in ATV susceptibility. Twenty-four (80%) of these I84V-containing isolates also contained an A71V or A71T mutation and had a 26.6 and 15.3 mean and median fold-change from reference in ATV susceptibility, respectively.
[2]The isolates containing the L90M mutation had an average 5.3-fold change and a median 2.2-fold change from reference in ATV susceptibility.

Of the 115 isolates from ATV treatment arms that had the L90M mutation at baseline, 23 (20%) of the isolates that contained the A71V mutation were responders while 85 (74%) were virologic failures or DC before suppressed compared to the 65 isolates from other treatment groups (SQV/RTV, LPV/RTV, or efavirenz) where 27 (42%) of the isolates that contained the L90M mutation were responders and 22 (43%) were virologic failures or DC before suppressed (Table H).

Of the 115 isolates from ATV treatment arms that had the A71V mutation at baseline, 32 (28%) of the isolates that contained the A71V mutation were responders while 72 (62%) were virologic failures or DC before suppressed compared to the 94 isolates from other treatment groups (LPV/RTV or SQV/RTV) where 53 (56%) of the isolates that contained the A71V mutation were responders and 31 (33%) were virologic failures or DC before suppressed (Table J).

Table J. Response of Antiretroviral-Experienced Patients with the A71V or N88 Mutation at Baseline

	A71V [1]		N88[2]	
	ATV	LPV/RTV or SQV/RTV	ATV	LPV/RTV or SQV/RTV
Virologic failures	37 (32%)	11 (12%)	22 (38%)	6 (11%)
DC before suppression	35 (30%)	20 (21%)	10 (18%)	4 (7%)
DC while suppressed	11 (10%)	10 (11%)	2 (4%)	7 (13%)
Responders	32 (28%)	53 (56%)	23 (40%)	38 (69%)
n	115	94	57	55

[2]The 112 isolates containing a N88 mutation had an average 3.4 fold-change and a median 2.3 fold-change from reference in ATV susceptibility.

Fifty-six percent of the isolates with an N88 substitution at baseline in an ATV treatment arm failed treatment or discontinued before suppression compared to 18% in the LPV/RTV or SQV/RTV treatment arms (Table J). Sixty-eight percent of the isolates with an M46I mutation at baseline in an ATV treatment arm failed treatment or discontinued before suppression compared to 47% in the LPV/RTV or SQV/RTV treatment arms (Table K).

Table K. Response of Antiretroviral-Experienced Patients with an M46I Mutation

	M46I	
	ATV	LPV/RTV or SQV/RTV
Virologic failures	23 (40%)	9 (21%)
DC before suppression	16 (28%)	11 (26%)
DC while suppressed	6 (10%)	1 (2%)
Responders	13 (22%)	21 (50%)
n	58	42

The 100 isolates containing a M46I mutation had an average 6.2 fold-change and a median 2.7 fold-change from reference in ATV susceptibility.

These data suggest that mutations L90M, I84V, A71V, M46I and substitutions at N88 are detrimental to ATV antiviral activity and may affect virologic response to ATV treatment clinically.

Response of antiretroviral-experience patients from study 043 with a D30N mutation

There were 222 patient isolates from study 043 with outcomes (98 patient isolates had no outcome) with 112 in the ATV arm and 110 in the LPV/RTV arm. The responders and virologic failures from this trial and the response based on the presence of the nelfinavir-associated D30N mutation was analyzed (Table L).

Table L. Response of Antiretroviral-Experienced Patients in Trial 043 with an D30N Mutation

	ATV	LPV/RTV
Total n	112	110
Responders, n (%)	80 (71%)	74 (67%)
Virologic Failures	26	28
With the D30N	27	20
Responders, n (% of total responders)	26 (33%)	15 (20%)
Virologic Failures	0	3
Without the D30N	85	90
Responders, n (% of total responders)	54 (67%)	59 (80%)
Virologic Failures	31	31

The majority of D30N-containing isolates responded overall – 96% (26/27) in the ATV arm and 75% (15/20) in the LPV/RTV arm. A higher percentage of D30N-containing isolates responded in the ATV arm (33%) than in the LPV/RTV arm (20%), while the isolates without D30N responded better in the LPV/RTV arm (80%) than in the ATV arm (67%). These data suggest that patients that have prior nelfinavir experience and have the D30N mutation in their HIV will respond well to ATV treatment. In addition, the data suggest that the relatively low PI-experience (50%) of the patient populations in Study 043 and 045 and the percentage of prior nelfinavir experience with D30N mutations at baseline (around 25%) in these trials may give a better response rate for ATV than will be seen in more highly PI-experienced patient populations.

6.2 Clinical cross-resistance

An examination of cross-resistance using the baseline genotypic and phenotypic data from all studies demonstrated that a high-level of cross-resistance exists between ATV and other PIs - indinavir, lopinavir, ritonavir, saquinavir and nelfinavir. Of the ATV-resistant isolates, amprenavir had the lowest median fold-change from reference (2.6-fold) followed by saquinavir with a 4.7-fold change (Table M). The median

fold-change for isolates resistant to each of the other PIs (amprenavir, indinavir, lopinavir, ritonavir, saquinavir, and nelfinavir) is greater than 2.5-fold indicating that ATV is cross-resistant with these PIs and may not be very effective against isolates in PI-experienced patients. Of the ATV-resistant isolates, 100% were also resistant to nelfinavir and a high percentage (51%-84%) were resistant to the other PIs with amprenavir having the lowest percentage (51%) of resistance (Table N).

Table M. Clinical Cross-Resistance By Phenotype* (Median fold-change from reference)

	ATV-Resistant N = 416	**APV-Resistant** N = 236	**IDV-resistant** N = 58	**LPV-Resistant** N = 285	**RTV-Resistant** N = 465	**SQV-resistant** N = 303	**NFV-resistant** N = 677
ATV	7.2	11.2	5.6	11	6	10.5	3.5
APV	2.6	5.5	2.5	4.1	2.5	3.5	1.5
IDV	9.3	15.9	9.8	nd	8.8	12.8	2.7
LPV	6.1	12	nd	11	6	8.1	2.4
RTV	17.8	37.5	17.9	33	16.8	24	5.6
SQV	4.7	10.2	2.1	8	3.6	9.6	2.1
NFV	24	28.6	16.1	22.5	14	29.6	17

*Baseline phenotypic data from all studies

Table N. Clinical Cross-Resistance By Phenotype* (% resistant)

	ATV-Resistant N = 416	**APV-Resistant** N = 236	**IDV-resistant** N = 58	**LPV-Resistant** N = 285	**RTV-Resistant** N = 465	**SQV-resistant** N = 303	**NFV-resistant** N = 677
ATV	**100%**	**91%**	**79%**	**87%**	**75%**	**95%**	**61%**
APV	**51%**	100%	50%	67%	50%	61%	34%
IDV	**79%**	97%	100%	nd	82%	82%	53%
LPV	**69%**	93%	nd	100%	70%	73%	49%
RTV	**84%**	98%	88%	99%	100%	92%	65%
SQV	**69%**	78%	40%	71%	60%	100%	45%
NFV	**100%**	97%	97%	97%	94%	99.7%	100%

*Baseline phenotypic data from all studies

An examination of cross-resistance by baseline genotype of isolates from all studies showed that isolates with I84V, G48V, L90M, V82A and I50L have median fold-changes in ATV susceptibility of greater than 2.5-fold and are cross-resistant (Table O). Isolates with these mutations may not respond effectively to ATV treatment. Isolates with D30N or I50V maintained a median fold change in ATV susceptibility below 2.5-fold suggesting these mutations remain susceptible to ATV. In isolates that contained mutations I84V or G48V, >90% were resistant to ATV (Table P). Greater than 60% of isolates containing L90M, a change at V82, A71V/T (62%) or M46I (69%) were resistant to ATV. Fifty-one percent of isolates containing a

N88S/D were resistant to ATV and 38% and 12% of isolates with mutations D30N and I50V were resistant to ATV, respectively. The I50V confers resistance to amprenavir but generally not to ATV. Isolates with the I50L mutation were resistant to ATV but all remained susceptible to the other PIs (amprenavir, indinavir, lopinavir, ritonavir, saquinavir, and nelfinavir).

Table O. Clinical Cross-Resistance By Genotype* (Median fold-change from reference)

	I84V n = 95	**D30N** n = 166	**G48V** n = 32	**L90M** n = 343	**V82** n = 285	**I50V** n = 8	**I50L** n = 25
ATV	13.8	2.1	14.2	5	5	1.1	8.7
APV	6.5	0.64	2.1	1.8	2.1	10.2	0.6
IDV	15.3	1.5	nd	10.4	9.2	nd	0.41
LPV	11.5	0.8	11.6	3	10	6.5	0.25
RTV	33	0.98	30	9.9	24.5	7.8	0.24
SQV	37	1.3	105	3.8	1.9	0.8	0.4
NFV	25	33	15	13	11	2.2	0.57

*Baseline genotype and phenotype data from all studies.

Table P. Clinical Cross-Resistance By Genotype* (% resistant)

	I84V n = 95	**G48V** n = 32	**L90M** n = 343	**V82** n = 285	**D30N** n = 166	**I50V** n = 8	**I50L** n = 20
ATV	**93%**	**94%**	**66%**	**64%**	**38%**	**12%**	**95%**
APV	84%	41%	41%	42%	5%	100%	**0%**
IDV	100%	nd	66%	74%	12%	nd	**0%**
LPV	89%	75%	54%	74%	7%	100%	**0%**
RTV	98%	94%	77%	83%	11%	87%	**0%**
SQV	94%	97%	60%	44%	20%	25%	**0%**
NFV	95%	100%	85%	79%	98%	50%	**0%**

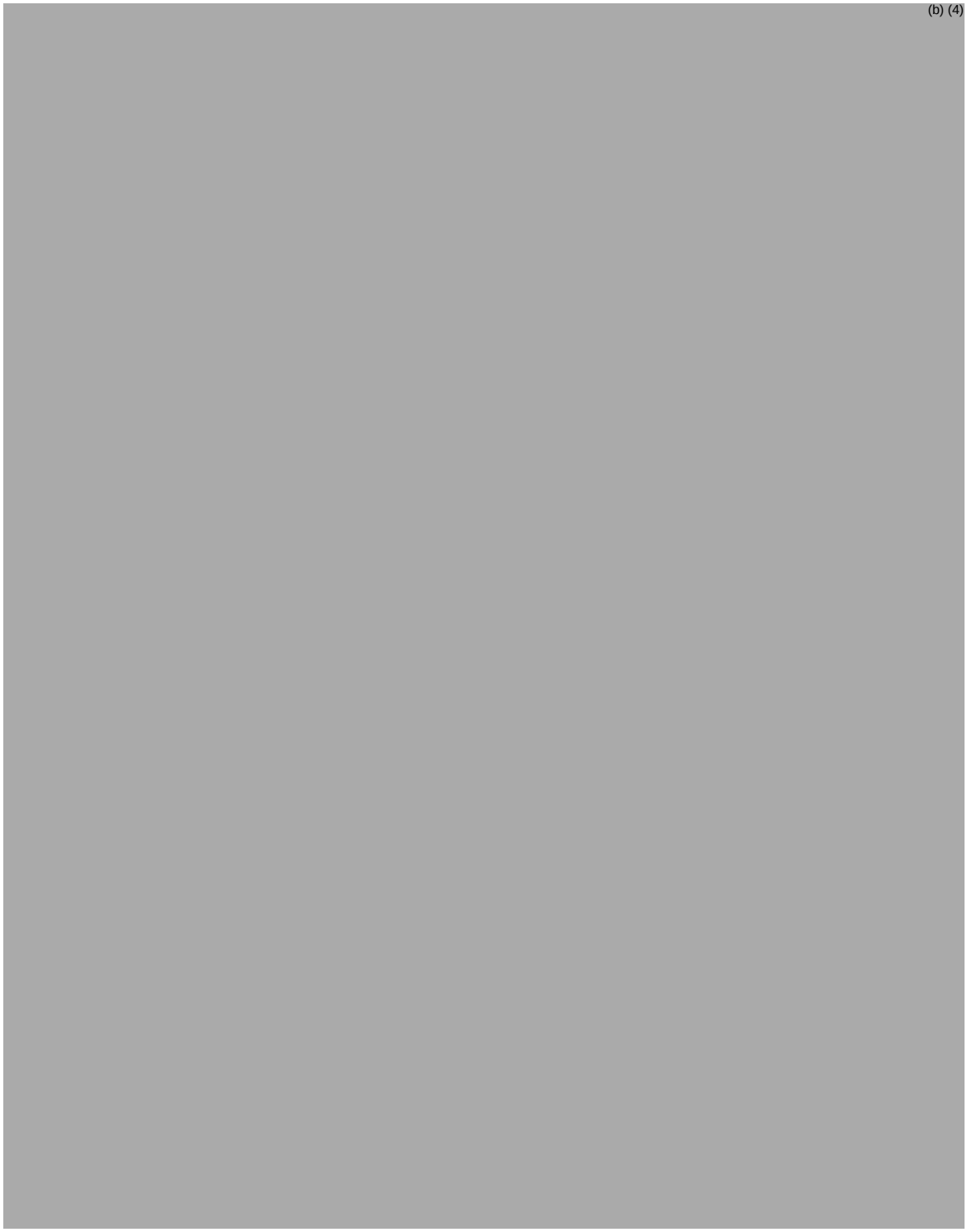
*Baseline genotype and phenotype data from all studies.

7.0 Conclusions

This NDA for atazanavir 100, 150 and 200 mg capsules is approvable with respect to microbiology for the treatment of HIV in combination with other anti-HIV agents. It offers a simple once-a-day regimen with the benefit of reduced lipid and triglycerides compared to lopinavir. ATV has a unique resistance profile in antiretroviral naïve patients with the development of the I50L mutation, which retains susceptibility to other protease inhibitors. In antiretroviral-treatment-experienced patients, the use of ATV should depend on ATV susceptibility determined by genotypic and/or phenotypic assays. ATV is highly cross-resistant with other protease inhibitors against HIV isolates from treatment-experienced patients.

8.0 Package Insert

(b) (4)



(b) (4)

DAVDP Finalized Clean Copy of Patient Insert

Microbiology

Mechanism of Action

Atazanavir is an azapeptide HIV-1 protease inhibitor. The compound selectively inhibits the virus-specific processing of viral Gag and Gag-Pol polyproteins in HIV-1 infected cells, thus preventing formation of mature virions.

Antiviral Activity *In Vitro*

Atazanavir exhibits anti-HIV-1 activity with a mean 50% effective concentration (EC_{50}) in the absence of human serum of 2 to 5 nM against a variety of laboratory and clinical HIV-1 isolates grown in peripheral blood mononuclear cells, macrophages, CEM-SS cells, and MT-2 cells. Two-drug combination studies with atazanavir showed additive to antagonistic antiviral activity *in vitro* with abacavir and the NNRTIs (delavirdine, efavirenz, and nevirapine) and additive antiviral activity *in vitro* with the protease inhibitors (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir) and NRTIs (didanosine, lamivudine, stavudine, tenofovir, zalcitabine, and zidovudine) without enhanced cytotoxicity.

Resistance *In Vitro*

HIV-1 isolates with reduced susceptibility to atazanavir (93- to 183-fold resistant) from three different viral strains were selected *in vitro* by 5 months. The mutations in these HIV-1 viruses that appeared to contribute to atazanavir resistance included N88S, I50L, I84V, A71V, and M46I. Changes were also observed at the protease cleavage sites following drug selection. The I50L substitution, with or without

an A71V substitution, conferred atazanavir resistance in recombinant viral clones in a variety of genetic backgrounds. Recombinant viruses containing the I50L mutation were growth impaired and showed increased susceptibility to other protease inhibitors (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir).

Cross-Resistance *In Vitro*

Atazanavir susceptibility was evaluated *in vitro* using a diverse panel of 551 clinical isolates from patients without prior atazanavir exposure. These isolates exhibited resistance to at least one approved protease inhibitor, with resistance defined as □2.5-fold change in EC_{50} relative to a reference strain. Greater than 80% of the isolates resistant to 1 or 2 protease inhibitors (with the majority resistant to nelfinavir) retained susceptibility to atazanavir despite the presence of key mutations (eg, D30N) associated with protease inhibitor resistance. Of 104 isolates displaying nelfinavir-specific resistance, 84 retained susceptibility to atazanavir. There was a clear trend toward decreased atazanavir susceptibility as isolates exhibited resistance to multiple protease inhibitors. Baseline phenotypic and genotypic analyses of clinical isolates from atazanavir clinical trials of protease inhibitor-experienced subjects showed that isolates cross-resistant to multiple protease inhibitors were also highly cross-resistant (61%-95%) to atazanavir. Greater than 90% of the isolates containing mutations I84V or G48V were resistant to atazanavir. Greater than 60% of isolates containing L90M, A71V/T, M46I, or a change at V82 were resistant to atazanavir, and 38% of isolates containing a D30N mutation in addition to other changes were resistant to atazanavir. Atazanavir-resistant isolates were highly cross-resistant (51%-100%) to other protease inhibitors (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir). The I50L and I50V substitutions yielded selective resistance to atazanavir and amprenavir, respectively, and did not appear to confer cross-resistance.

Resistance *In Vivo*

Atazanavir-resistant isolates have been obtained from patients experiencing virologic failure on atazanavir therapy. There were 14 atazanavir-resistant isolates from studies of treatment-naive patients (n=96 evaluable isolates) that showed decreases in susceptibility levels from baseline, and all had an I50L substitution emerge on atazanavir therapy (after an average of 50 weeks of therapy) often in combination with an A71V mutation. Phenotypic analysis of the isolates containing the signature mutation I50L showed atazanavir-specific resistance, which coincided with increased susceptibility to other protease inhibitors (amprenavir, indinavir, lopinavir, nelfinavir, ritonavir, and saquinavir). In contrast, 89% (32 of 36) of atazanavir-resistant isolates from studies of treatment-experienced patients (n=67 evaluable isolates) treated with atazanavir (n=26) or atazanavir plus saquinavir (n=10) showed no evidence of the emergence of the I50L substitution. Instead, these isolates displayed decreased susceptibility to multiple protease inhibitors and contained mutations associated with resistance to multiple protease inhibitors. These mutations included I84V, L90M, A71V/T, N88S/D, and M46I, which conferred atazanavir resistance and reduced the clinical response to atazanavir. Generally, if protease inhibitor mutations were present in the HIV-1 of the patient at baseline, atazanavir resistance developed through mutations associated with resistance to other protease inhibitors instead of the I50L mutation. These mutations conferred high cross-resistance to other protease inhibitors with 100% of

the isolates resistant to nelfinavir, >80% of the isolates resistant to indinavir, ritonavir, and saquinavir, and >35% of the isolates resistant to amprenavir and lopinavir. Genotypic and/or phenotypic analysis of baseline virus may aid in determining atazanavir susceptibility before initiation of atazanavir therapy.

9.0 Recommendations

9.1 Recommendations

Phase IV Commitments:

1. Submit analysis of protease cleavage sites in ATV- resistant patients from studies 034, 043 and 045 by 2Q04.

2. Follow a cohort of patients who failed on ATV treatment and developed the I50L mutation on new physician-selected PI regimens for 48 weeks compared to an NNRTI-failure/PI- naïve patient cohort and determine treatment response, baseline genotypes and phenotypes, and genotypes and phenotypes of virologic failures. Protocol should be submitted by 1Q04.

3. Test the activity *in vitro* of atazanavir against multiple clinical isolates of non-clade B subtypes of HIV-1 and HIV-2.

10.0 Appendixes

1. Listing of Isolates from Patients on ATV-treatments that Experienced Virologic Failure from Studies of Antiretroviral Therapy-Naive Subjects
2. Listing of Isolates from Patients on ATV-treatments that Experienced Virologic Failure from Studies of Antiretroviral Therapy-Experienced Subjects

11.0 References

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APPENDIX 1.

Listing of Isolates from Patients on ATV-treatments that Experienced Virologic Failure from Studies of Antiretroviral Therapy-Naïve Subjects

	PROT	PID	TRT arm	WK	OUTCOME	I50	A71	M46	ATV
1	AI424-007	00010 00020	ATV 200	33.42857	Virologic failure	L	A/V	M/I	4.85124
2	AI424-007	00020 00347	ATV 500	80.57143	Virologic failure	L	V	M/L	10.691
3	AI424-007	00020 00398	ATV 400	69.42857	Virologic failure	L			8.83864
4	AI424-007	00040 00450	ATV 200	25.71429	Virologic failure	L	V		7.92609
5	AI424-007	00045 00296	ATV 400	78.71429	Virologic failure	L	V		9.9449
6	AI424-034	00040 00486	ATV 400	49.14286	Virologic failure	L	A/V		5.8
7	AI424-034	00043 00226	ATV 400	54	Virologic failure	L	A/V		3.5
8	AI424-034	00067 00562	ATV 400	60	Virologic failure	L	V		29
9	AI424-034	00076 00586	ATV 400	48.14286	Virologic failure	L			8.6
10	AI424-034	00094 00772	ATV 400	42.85714	Virologic failure	L			5.2
11	AI424-008/AI424-044	00060 00076	ATV 400	12.14286	Virologic failure	L			16
12	AI424-008/AI424-044	00060 00169	ATV 400	90	Virologic failure	L			12
13	AI424-008/AI424-044	00060 00510	ATV 400	80	Virologic failure	L	A/V		6.7
14	AI424-007/AI424-041	00050 00096	ATV 400	172	Virologic failure	L	A/V		19

APPENDIX 2.

Listing of Isolates from Patients on ATV-treatments that Experienced Virologic Failure from Studies of Antiretroviral Therapy-Experienced Subjects

	PROT	PID	TRT ARM	WK	PR Mutations	ATV IC_{50}
1	AI424-009	00004 00077	ATV 400/SQV	38	A71V, L90M	2.97
2	AI424-009	00007 00064	ATV 400/SQV	56	N88D, **L90M, I84V, M46I**	22.00
3	AI424-009	00008 00089	ATV 400/SQV	24.8	N88D, A71T, M46I, **I84V**	11.64
4	AI424-009	00012 00015	ATV 400/SQV	23.9	A71V, N88D, **I84V**	48.77
5	AI424-009	00012 00047	ATV 400/SQV	63	N88S, **A71V/T, I84V, M46I**	140.76
6	AI424-009	00014 00023	ATV 400/SQV	63	N88S, **A71T, I84V, M46I**	30.66
7	AI424-009	00017 00005	ATV 400/SQV	24	N88D	7.41
8	AI424-009	00017 00078	ATV 400/SQV	23.71	N88D, A71T, L90M	14.37
9	AI424-009	00019 00065	ATV 400/SQV	62.7	L90M, M46I, **A71V**	6.93
10	AI424-009	00045 00072	ATV 400/SQV	67	**L90M, N88S, A71V**	3.60
11	AI424-043	00051 00160	ATV 400	24	A71V, I84V, L90M	14
12	AI424-043	00004 00036	ATV 400	19	N88S, M46I	13.28
13	AI424-043	00033 00122	ATV 400	17	L90M, A71V/T	4.06
14	AI424-043	00036 00037	ATV 400	12	L90M, M46I	15.59
15	AI424-043	00051 00035	ATV 400	24	L90M, **I84V, A71T**	16.33
16	AI424-043	00065 00238	ATV 400	24	L90M, M46I	6.6
17	AI424-043	00065 00318	ATV 400	16	N88S	13.33
18	AI424-043	00065 00323	ATV 400	16	L90M	14.93
19	AI424-043	00070 00010	ATV 400	16	N88D	8.46
20	AI424-043	00065 00321	ATV 400	16	A71V, L90M	19
21	AI424-043	00086 00062	ATV 400	16	A71V, I84V, L90M	35.34
22	AI424-043	00086 00078	ATV 400	16	N88D, **A71V**	10.32
23	AI424-043	00099 00391	ATV 400	24	D30N, **A71T,** N88D	11
24	AI424-043	00098 00206	ATV 400	16	A71V, L90M	2.81
25	AI424-043	00105 00354	ATV 400	16	A71V, N88D, M46I, L90M	4.98
26	AI424-043	00117 00082	ATV 400	17	M46I, L90M	6.40
27	AI424-043	00096 00359	ATV 400	24	**A71V**	108.00
28	AI424-043	00098 00455	ATV 400	12	L90M, **I84V**	12.00
29	AI424-043	00097 00357	ATV 400	10	**A71T, N88D**	9.60
30	AI424-043	00062 00412	ATV 400	24	A71T, L90M, M46I	7.90
31	AI424-043	00061 00350	ATV 400	32	**N88S**, M46I	8.00
32	AI424-043	00062 00258	ATV 400	24	A71V, L90M	7.30
33	AI424-043	00012 00219	ATV 400	42	D30N, M46I, N88D **A71T, I50L**	57
34	AI424-043	00062 00382	ATV 400	34	A71V, L90M**, I50L, I84V**	23
35	AI424-043	00063 00129	ATV 400	36	**I50L, N88T**	3.5
36	AI424-043	00065 00329	ATV 400	24	D30N, M46I, N88D, **I50L**	15

Mutations that developed are in bold.

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